

Nicotine Addiction and Impulsive Behaviour: Disentangling the Relationship

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Drug addiction can now be considered a global epidemic with considerable psychological, physical, social and economic costs. Substantial research remains focused upon furthering our understating of the disorder, for which current treatments are limited in their effectiveness. More recently there has been an increased interest in the multidimensional construct of impulsivity and its association with addiction. Impulsivity is a hallmark feature of drug addiction, with drug users frequently displaying a preference for immediate over delayed gratification (impulsive choice) and a loss of inhibitory control (disinhibition) (Madden et al., 1997). To date, our understanding of the complex association between impulsivity and drug addiction has been hindered by the cross sectional nature of the majority of research conducted. As a result of this it has been unclear whether impulsivity is a risk factor or a consequence of drug abuse. The experiments of this thesis were primarily concerned with exploring the latter of these theories, in the hope of elucidating the role of drug induced impulsivity in the establishment, maintenance and relapse of drug dependence. This was achieved by exploring the effects of nicotine in two animal paradigms of impulsivity; the delayed reward paradigm which assessed impulsive choice, and the symmetrically reinforced go/no-go visual discrimination task which measured behavioural disinhibition. A series of preliminary experiments confirmed the suitability of both tasks for exploring the relationship between impulsivity and nicotine dependence. Behavioural disinhibition in the go/no-go task was stable, lacked sensitivity to changes in primary motivation and furthermore appeared not to be dependent on timing mechanisms. Impulsive choice in the delay discounting task was stable and delay sensitive. Whilst decreasing primary motivation was without effect on impulsive choice, increasing motivation for food reward was found to reduce levels of impulsivity. Acutely, nicotine increased both disinhibition (0.5mg/kg, s.c.) and impulsive choice (0.125, 0.25, 0.5mg/kg, s.c.). The acute effects of nicotine on both components of impulsivity were effectively antagonised by the centrally acting antagonist mecamylamine, which alone was without effect in either model. Adopting a longitudinal design, the effects of chronic nicotine administration (3.16mg/kg/day for seven days, osmotic mini pumps), nicotine withdrawal and the residual sensitivity to nicotine following a sustained period of abstinence were then explored. Chronic nicotine administration enhanced disinhibition and impulsive choice, an effect that was greatest at the initial stages of treatment, suggesting that drug tolerance may have developed. Nicotine withdrawal had differential effects on impulsive choice and disinhibition. In the delay discounting task both initial and long-term withdrawal was associated with enhanced sensitivity to delayed reward. This effect, however, was restricted to low “trait” impulsive animals. Conversely, initial withdrawal induced a short-lived rebound increase in inhibitory control, following which a gradual decrease in inhibitory control was observed that reached significance during the second week of withdrawal. Evidence that the effects of nicotine on impulsivity were temporary was shown, with behaviour returning to base line levels three weeks following termination of treatment. Finally, acute nicotine challenges (0.125, 0.25, 0.5mg/kg, s.c.) demonstrated that chronic nicotine exposure rendered animals hypersensitive to the effects of nicotine on disinhibition. Overall the present experimental investigations provide evidence to suggest that impulsivity may be a key component at both the initial and end stages of addiction. Behavioural and pharmacological treatments that target impulsivity may prove to be effective future treatments for the disorder.

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Abbreviations

ACC	anterior cingulate cortex
ANOVA	analysis of variance
ANCOVA	analysis of covariance
APD	antisocial personality disorder
BIS	Barrat Impulsivity Scale
BL	baseline
BLA	basolateral amygdale
BS	between subjects
BW	bodyweight
CDR	choice of delayed reward
CRF	continuous reinforcement
CSF	cerebrospinal fluid
CPT	continuous performance task
DA	dopamine
DD	delay discounting
Df	degrees of freedom
DHβE	Dihydro-β-erthyroidine
DRL	Differential Reinforcement of Low Rate Procedure
EDT	Experiential Discounting Task
EQ	Eysenck Impulsivity Questionnaire
EIVE	Eysenck Impulsiveness and Venturesomeness-Empathy Questionnaire
FC	frontal cortex
5CSRTT	Five Choice Serial Reaction Time Task
5-H1AA	5-hydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
FCN	fixed consecutive number
fMRI	functional magnetic resonance imaging
GABA	gamma aminobutyric acid
HI	high impulsive
i.m.	Intramuscular
ILC	infralimbic cortex
i.p.	intraperitoneal
ITI	inter-trial-interval
kg	kilogram
LD	light/dark
LE	Long Evans
LH	Lister Hooded
LI	low impulsive
M	mixed
MANOVA	multivariate analysis of variance
MAO	monoamine oxidase
MD	mixed design
mg	milligram
ml	millilitre
N	nicotine
NAc	nucleus accumbens
N.S.	non-significant
OFC	orbitofrontal cortex
mPFC	medial prefrontal cortex
nAChRs	nicotinic acetylcholine receptors
PCPA.	p-chlorophenylalanine
%	percentage
PFC	prefrontal cortex

PLC	prelimbic cortex
±	plus or minus
RM	repeated measures
RT	reaction time
RTD	rapid tryptophan depletion
S	saline
s.c.	subcutaneous
SD	Sprague Dawley
SEM	standard error of the mean
5-HT	serotonin
5-HTT	serotonin transporter
STN	subthalamic nucleus
SPSS	Statistical Package for Social Sciences version 14.0
SSRI	Selective serotonin reuptake inhibitors
SSRT	stop signal reaction time
SST	stop signal task
THC	Tetrahydrocannabinol
UK	United Kingdom
VS	ventral striatum
VTA	ventral tegmental area
WHO	World Health Organisation
W	Wistar

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The Relationship Between Impulsivity and Nicotine Dependence: Where do we go next? Institute of Psychological Sciences, University of Leeds, June 2003.

CHAPTER 1

Impulsivity and drug addiction: Review and integration of the human and animal literature

1.1. DRUG ADDICTION

Drug addiction is now considered a global epidemic. It is a deeply complex disorder that has profound consequences at the psychological, physical, social and economic level (Altman et al., 1996). Current estimates suggest that approximately 34.1% of the adult population within the United Kingdom (UK) has sampled one or more illicit drugs in their lifetime, while 2.5 million of these individuals are believed to currently meet the diagnostic criteria for dependence (Office of National Statistics, 2005; British Crime Survey, 2006). In terms of licit addictive agents, it is believed that 25% of the UK population are currently smokers and 7% of the population are currently dependent on alcohol. At an individual level, substance misuse is associated with significant morbidity and mortality while the costs to society are substantial. Including both alcohol and nicotine, a staggering 124,000 deaths a year in UK are drug related, while the annual economic impact arising from health care costs and drug related crime is in excess of 18 billion pounds (Office of National Statistics, 2005).

The considerable burden of drug addiction and dependence on society has meant significant research effort remains focused upon extending our understanding of the neurobiological and psychological mechanisms underlying the disorder. With current treatments for the disorder being limited in their effectiveness, such research is crucial for the development of more successful treatment and prevention strategies (Nestler et al., 2003).

1.1.1. Theories of Drug Addiction

Conceptualisation and knowledge of addiction is evolving rapidly. Despite this, the precise definition of the disorder remains controversial. Earlier attempts encompassed both tolerance and withdrawal as major features of drug addiction (Kalant, 1987). Both these physiological phenomena however can occur without addictive behaviour (O'Brien and Volkow, 2006), leading to more recent definitions focusing on the more behavioural aspects of the disorder. Addiction typically involves the uncontrollable compulsive pattern of drug-seeking and drug-taking behaviour that takes place at the expense of most other activities and in the face of damaging health and social consequences (Robinson and Berridge, 2003; West, 2006). It is important to recognise that casual drug taking does not always lead to the progression of

addiction. Drug addiction, it seems, arises as a result of complex interactions between drug effects, environmental and neurobiological factors (e.g. Meaney, Brake and Gratton, 2002).

Earlier theoretical explanations of addiction focused upon the drive to experience the positive affective states associated with the use of the abused drugs as a key mechanism underlying the disorder (e.g. Markou et al., 1993). The ability of addictive drugs to produce euphoria through activation of the brains natural reward circuitry has been the focus of much theory and research in attempt to further understanding of addiction. Despite drugs of abuse such as cocaine, amphetamine, opioids, nicotine, alcohol and marijuana having varying initial targets, their common ability to enhance extracellular levels of dopamine (DA) in the nucleus accumbens (NAc) and related areas of the mesolimbic-mesocortical DA pathway is believed to be critical for their acute reinforcing actions (e.g. Koob and Bloom, 1988; Robbins and Everitt, 1996; Everitt et al., 2001; Nestler, 2005). It is argued that once the rewarding properties have been experienced this promotes repeated use of the addictive drug. While it is highly probable that initial drug use may be associated with their euphoric and pleasurable effects, and that in some instances addicted individuals abuse drugs solely to achieve these positive effects, the theory cannot however account for the continuation of drug abuse when the pleasurable effects are minimal or not present (e.g. Lamb et al., 1991; Robinson and Berridge, 1993).

Further theories have instead focused on the avoiding of aversive withdrawal in an attempt understand the behavioural and neurobiological processes involved in drug dependence and addiction. Withdrawal is believed to arise as a consequence of chronic drug use. Repeated drug exposure is known to produce complex neuroadaptations of the DA system in an attempt to maintain internal homeostasis in response to the over activation caused by addictive drugs (eg. Koob and Le Moal, 2001). A common disruption across drug addictions is the observed DA hypoactivity (e.g. Dakis and O'Brien 2005), believed to be associated with the withdrawal experienced following termination of use or between drug intakes (Altman et al., 1996). Drug withdrawal is associated with a number of aversive symptoms both emotional and physical (Hughes et al., 1984; Hughes et al., 1991). The avoidance of these symptoms is argued to be a powerful motivation to continue to abuse drugs. However, as previously discussed, the avoidance of withdrawal alone as an explanation of addiction fails to account for the dependence of substances, such as amphetamine, that do not induce a withdrawal syndrome following termination of use. More importantly, this explanation of the disorder cannot account for relapse into drug taking that can occur months or even years after abstinence by which time the symptoms of withdrawal, at least in terms of physical signs, have long dissipated (Robinson and Berridge, 2000).

The failure of these theories to account for the complexity of addiction has prompted researchers to explore further the neurobiological and psychological mechanisms of disorder. The incentive-sensitisation theory proposed by Robinson and Berridge (1993) is an example of such a theory. Central to theory is that with chronic drug exposure neuroadaptations are produced that sensitise DA and other neurotransmitter systems in the NAc circuitry. This in turn is believed to cause an enhancement of neurotransmission in response to both the drug of abuse and drug related stimuli (Robinson and Berridge, 2000; 2003). The neuroadaptations within this system are independent from those mediating drug induced pleasure and withdrawal and are argued instead to underlie the incentive salience or 'wanting' aspect of reward (Robinson and Berridge, 2000). With repeated drug exposure, incentive value becomes associated to drug related stimuli making them increasingly 'wanted' leading to intense drug craving and ultimately compulsive drug seeking and taking behaviour in their presence. These neuroadaptations have furthermore been found to persist long after termination of drug exposure in both humans and animals (e.g. Strakowski et al., 1996; Castner and Goldman-Rakic 1999). This persistent sensitisation therefore renders previously dependent individuals susceptible to relapse long after drug cessation.

This theory of addiction and relapse has received extensive support both in the human and animal literature (Robinson and Berridge, 2000; 2003). Whilst the model effectively provides a theoretical account of the compulsive drug use as well as the involuntary nature of craving associated with addiction, it fails however, to adequately encompass the loss of control over behaviour that is additionally observed in this population (Goldstein and Volkow, 2002). Drug dependent individuals frequently display a loss of control over drug intake and fail repeatedly in their ability to successfully abstain despite the potentially harmful consequences. More recent theories of addiction have therefore begun to focus on inhibitory control, or impulsivity, in an attempt to understand further the disorder of drug addiction. Considerable evidence from neuroimaging studies has demonstrated that chronic drug abusers display abnormalities in both the structure and function of the prefrontal cortex in regions of the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) (e.g. Franklin et al., 2002; Goldstein and Volkow, 2002; Rose et al., 2003). These regions are known to be involved in decision making, the processing of affective and reward value of stimuli (OFC) and the inhibitory control over behaviour (ACC) (e.g. Aron et al., 2004).

Jentsh and Taylor (1999) were amongst the first to hypothesise that chronic drug abuse is associated with dysfunctions of the PFC, thereby leading to a loss of control of future drug use and the maintenance of addiction. More specifically, it was proposed that long term drug exposure leads to impaired inhibitory control over the appetitive responses elicited by the abused drug and drug related stimuli, thus increasing the likelihood of drug relapse. More

recently Goldstein and Volkow (2002) have described addiction as a disorder of impaired response inhibition and salience attribution (I-RISA). The I-RISA model of addiction postulates that the disorder is associated with increased salience of drug rewards and associated stimuli, decreased salience of alternative natural reinforcers, and weakened inhibitory control over responding to drug related stimuli (Goldstein and Volkow, 2002). Comparably Lubman et al. (2004) conceptualise the syndrome of addiction as ‘compulsive’, arguing that the loss of control over drug use and continual drug seeking and taking behaviour is mediated by the impaired prefrontal cortex circuitry which gives rise to a disorder of inhibitory dysregulation.

Yet to be fully established by these theoretical accounts of addiction, is whether the observed abnormalities in the PFC circuitry, which lead to impairments in inhibitory control, reflect a vulnerability to the development of addiction or instead are a consequence of chronic drug exposure (Goldstein and Volkow, 2002; Olmstead 2006). Despite this, what these models have successfully achieved is to extend traditional theories of addiction to incorporate impulsivity as a key feature of the disorder. The recent acknowledgement of the critical role of inhibitory control in addiction has led to an increased interest in the multidimensional construct of impulsivity and its association with drug dependence. It is the relationship between impulsivity and drug dependence that is the focus of the current thesis.

1.2. IMPULSIVITY: A MULTIDIMENSIONAL CONCEPT

Impulsivity is a concept that plays a major role in normal human behaviour and as Evenden (1999) states “adds important colour to everyday life.” Actions of impulsivity in everyday existence are relatively easy to identify e.g. spur of the moment purchasing or taking the risk of having an extra drink. For theorists however, definition of the concept is not an easy task and there is limited consensus over a precise definition. A number of characterisations of impulsivity have been proposed. Impulsivity has been equated to; the inability to sustain attention (Dickman, 1993), as part of the personality dimension of extraversion, (Eysenck, 1983), a tendency to engage in risky behaviours (Eysenck, 1993), and the seeking of novel sensations (Zuckerman, Eysenck, Eysenck, 1978). It has furthermore been described as an act of behaviour which is spur of the moment and lacking adequate attention (Barrett and Patton 1983), the propensity to engage in rapid information processing which is error-prone (Dickman, 1990) and the preference for immediate over delayed gratification (Rachlin and Green, 1972; Logue, 1988). From these diverse definitions what becomes clear is that impulsivity is not a unitary behaviour, but instead a multidimensional concept incorporating a variety of related phenomena of impulsiveness, that moreover may be mediated by contrasting neurobiological mechanisms (Hollander and Stein 1995; Evenden, 1999a). In an attempt to incorporate the variety of features of impulsivity within its definition, Moeller and colleagues (2001) stated that impulsivity is “a predisposition toward rapid, unplanned reactions to internal or external stimuli

without regard to the negative consequences of these reactions to the impulsive individual or to others”.

Impulsivity has been greatly studied both as a normal personality trait and as a dysfunctional behaviour (Stein et al., 1993). While many personality theorists consider impulsive behaviour to be a basic measurable dimension of personality (e.g. Barratt, 1985; Dickman, 1993; Eysenck, 1993), impulsivity in its pathological form is a clinical symptom in a diverse number of psychiatric illnesses. The syndromes in which impulsive behaviour is most associated with are substance abuse disorders, conduct disorder and its adult variant antisocial personality disorder, borderline personality disorder (e.g. Barratt et al., 1997; Links et al. 1999; Casillas and Clark, 2002), mania (e.g. Harmon-Jones et al., 1997; Swann et al., 2001) and attention deficit/hyperactivity disorder (e.g. Tannock et al., 1989; Sunohara et al., 1999; Solanto et al., 2001; Salbach et al., 2002). Furthermore, disorders such as kleptomania, pyromania and pathological gambling have been classified within the DSMIV as ‘impulsive control disorders’.

1.3. SUBSTANCE ABUSE AND IMPULSIVITY

Impulsivity is a hallmark feature of substance abuse disorders. According to the diagnostic criteria of the DSM IV the disorder can be defined as a dysfunction of impulsive control. Substance abuse can be conceptualised as a problem of impulsivity in that firstly drug dependent individuals frequently display a failure in the ability to refrain from inappropriate drug seeking and taking behaviour, continuing to administer the drug despite repeated efforts to abstain. Furthermore, they display an incapacity to wait, a change in the perception of time, and predominately select the more immediate drug rewards or the relief of drug withdrawal over various prosocial, often larger delayed rewards associated with a drug free lifestyle (Madden et al., 1997). The fact that impulsiveness is a prominent feature within substance abuse disorders has increasingly led research to focus on the possible role of the construct in the initiation, maintenance and relapse of the disorder. There are two broad approaches to human research exploring the relationship between impulsivity and substance abuse. They differ in techniques utilised to assess impulsivity using either; (i) self report personality questionnaires or (ii) behavioural state measures.

1.3.1. Impulsivity: Trait Approaches

Personality assessments of impulsivity are a widely used to explore impulsiveness in drug dependent populations. The most commonly adopted self-report measures include the Barratt Impulsivity Scale (BIS), Eysenck Impulsivity Questionnaire (EIQ), and the Eysenck Impulsiveness-Venturesomeness-Empathy questionnaire (EIVE).

The BIS (Barratt, 1985) has three subscales which each measure independent aspects of impulsivity; 'non-planning', or lack of future orientation, 'cognitive impulsiveness', or rapid, unstable thoughts and lack of cognitive patience, and 'motor impulsiveness', or a tendency to act recklessly. Studies adopting this scale have reported higher levels of trait impulsivity in substance abusers in comparison to matched controls (e.g. Patton, Stanford and Barratt, 1995; Swann, Dougherty, Pazzaglia, et al., 2004). More specifically individuals that are dependent on cocaine (Moeller et al., 2002; Patkar et al., 2002; Coffey et al., 2003), opioids (Kirby et al., 1999), alcohol (Soloff, Lynch and Moss, 2000; Bjork et al., 2004) and nicotine (Mitchell, 1999; Heyman and Gibb, 2006) have consistently demonstrated higher scores of impulsivity on the BIS relative to non-abusers. Furthermore, scores of impulsivity on the BIS appear to be associated with substance use severity. Levels of trait impulsivity have been found to positively correlate with the number of cigarettes smoked in males (Skinner, Aubin and Berlin, 2004), frequency of past alcohol consumption (Fishbein and Reuland, 1994), daily cocaine use (Moeller et al., 2001) and scores on the addiction severity index in polysubstance abusers (Reilly, Roll and Downey, 2000).

By utilising this scale, the importance of focusing on impulsivity in drug dependence treatment has also been highlighted. Smokers scoring higher on the BIS tend to relapse more rapidly following a one day cessation work shop (Doran et al., 2004), and more impulsive cocaine dependent people remain in a twelve week treatment programme for a significantly shorter period (Moeller et al., 2001).

Moreover, the BIS is able to differentiate between schizophrenic patients with or without substance abuse disorder (Blanchard et al., 2001; Dervaux et al., 2001; Gut-Fayand et al., 2001). Comparably, higher scores on impulsivity have additionally been observed in substance abusing pathological gamblers in comparison to non-abusing gamblers (Petry, 2001b). The finding that both gambling groups also differed from control subjects on impulsivity, suggests that perhaps an additive effect of substance abuse on self-reported trait impulsivity may be present in diagnosed gamblers.

The EIQ is an adapted subscale from the Eysenck Personality questionnaire (Eysenck and Eysenck 1978). Comparable reports of heightened trait levels of impulsivity have also been reported with this scale. Students that reported greater use of substances, including alcohol and cannabis, displayed higher scores on the scale in comparison to students that reported less frequent use (Golding and Cornish, 1987). In female social drinkers a positive correlation was furthermore shown between level of alcohol consumption and impulsivity score (Grau and Ortet, 1999). In dependent users, alcoholics, cocaine and opioid abusers have scored higher on impulsivity than matched controls on this measure (Madden et al., 1997; Conrod, Pihl, Stewart

and Dongier, 2000; Petry, 2001). The scale was furthermore able to differentiate between smokers, non-smokers (Bickel, et al., 1999; Mitchell, 1999,) and ex-smokers (Bickel et al., 1999) on levels of impulsivity, with smokers being significantly more impulsive.

The EIVE questionnaire (Eysenck and Eysenck 1978; Eysenck, Pearson, Easting and Allsopp, 1985) has three subscales; impulsivity, venturesomeness and empathy. The impulsivity scale is reflective of a general tendency toward lack of planning, quick decision making and impulsive action. Studies adopting this self report measure have demonstrated that polydrug use is associated with higher scores of impulsiveness in comparison to matched controls (Parrott, Sisk and Tuner, 2000; Butler and Montgomery, 2004). Research has in addition shown that opioid dependent individuals score significantly higher on the impulsiveness subscale relative to matched controls (Kirby et al., 1999), although to a lesser extent than inner city crack cocaine users (Lejeuz, Bornovalova, Daughters and Curtin, 2005). The scale has also been able to differentiate between heavy ecstasy users and non ecstasy users (Morgan 1998; Parrot et al., 2000) and high and low consumers of caffeine (Jones and Lejeuz, 2005), with heavier users of both drugs displaying significantly higher trait levels of impulsivity. Among substance abusers, higher levels of impulsivity on this scale have been associated with greater severity of use and dependence. More specifically, impulsivity scores correlate positively with abuse symptoms in methamphetamine users (Simons et al., 2005) and polydrug abusers score significantly higher than single drug users (McGowan, 1988).

Finally, EIVE has demonstrated that substance abuse disorder has an additive effect on measures of impulsivity in patients with co-existing psychopathologies, including suffers of pathological gambling (Petry, 2001), antisocial personality disorder (Petry, 2002) and bulimia (Kane, Loxton, Staiger and Dawe, 2004). Whilst patients of these disorders display heightened scores in comparison to controls, when co-morbid with substance abuse more extreme scores are demonstrated.

Trait measures of impulsivity have provided substantial evidence of heightened self reported impulsivity, a finding common across abusers of varying drugs. These studies however are unable to disentangle the causal relationship between impulsivity and substance abuse and dependence. These measures assess trait impulsivity, argued to be a relatively stable trait. It is therefore plausible that heightened impulsivity may develop early in ones life and can thus be associated with a susceptibility to substance abuse. It could however, be the case that impulsivity is less stable than originally theorised, and is instead a consequence of substance abuse. Support for the former theory is demonstrated from indications of continued heightened impulsivity following discontinuation of abuse in previous substance abusers (e.g. Allen, Moeller, Rhoades, Cherek, 1998; Blanchard et al., 2001). However, no definitive conclusions

can be made due the possibility that previous chronic exposure to drugs and alcohol could have increased impulsivity levels permanently in these subjects.

1.3.2. Impulsivity: Behavioural Approaches

Behavioural measures of impulsivity can be classified into two broad categories. Each category attempts to measure a distinctive component of impulsivity and represents an independent operational definition of the construct (White et al., 1994; Kindlon et al., 1995; Ho et al., 1999; McDonald et al., 2003; Reynolds et al., 2006a; Winstanley, Eagle and Robbins, 2006; Dom et al., 2007). The first are laboratory measures that define impulsivity as a failure in inhibitory control. The second are measures that characterise impulsivity as a preference for immediate over delayed gratification, even during instances when the immediate reward is less valuable, or smaller than the delayed reward. A range of tasks have been devised to measure each of the two dimensions of impulsivity, many of which have been modelled in animals with some success. These animal models of impulsivity provide the opportunity to explore the neurobiological mechanisms modulating impulsivity and its association with addiction (Olmstead, 2006).

1.3.2.1. Behavioural Tasks Measuring Inhibitory Control

Behavioural inhibition has been defined as the ability to appropriately withhold or terminate thoughts and actions (Logan, Cowan and Davis, 1984). As previously discussed a dysfunction in this ability has been argued to be a core component of addiction (Jentsch and Taylor, 1999; Lyvers, 2000; Goldstein and Volkow, 2002). Readily observed in abusers is the apparent loss of control over compulsive drug seeking and taking behaviour despite awareness of the negative consequences. An inability to effectively control one's behaviour thus leads to the continual failure of chronically addicted individuals to cease or regulate drug intake.

One of the most common tasks adopted in both humans and animals to assess inhibitory control is the go/no-go paradigm. Successful performance on the task requires subjects to inhibit prepotent behaviours (Newman and Kosson 1986). Subjects learn, by a process of trial and error, to respond as quickly as possible (normally made by a key press, or lever press in the case of animals subjects) to a particular discriminative stimulus, such as, a symbol displayed on a computer screen, a light or a tone. Correct responses to the 'Go signal' are normally rewarded and for humans can be in the form of small monetary rewards (e.g. gain £0.10), points or social rewards. In the case of food restricted animals' food rewards are utilised. Occasionally during the task subjects are required to inhibit a response when the 'Go signal' is absent and instead a 'No-go signal' is presented. In human versions of the task, dependent on the variant of the paradigm being utilised, incorrect responses during the presentation of the 'No-go signal' can lead to either no reinforcement, or punishment, through the reduction of reward (e.g. loss £0.10). Comparably, both asymmetrical and symmetrically reinforced go/no-go paradigms have

been developed with the use of animals. In the latter of the models, successful inhibition of responding, in addition to correct go responding during No-go trials, is also reinforced (e.g. Harrison, Everitt and Robbins, 1999). Across all variants of the task that have been developed, inappropriately responding during the absence of a 'Go signal', is argued to be a valid index of degree of impulsiveness (Newman and Kosson, 1986). These inappropriate responses are commonly known as errors of commission or false alarms.

Within the human literature a second commonly utilised paradigm that attempts to measure behavioural inhibition is the stop signal task (SST) (Logan, Schachar and Tannock, 1997). More complex than the aforementioned task, the task is based on the theory that inhibited behaviour is governed by two systems; one which activates and one which inhibits behaviour (Gray, 1976; Logan and Cowan, 1984; Fowles, 1987). A loss of control over behaviour can be the cause of either an over stimulated activation system or a weakened inhibitory system. The task measures inhibitory control by modelling the net effect of the counteracting activational and inhibitory systems, through the measurement of the participant's ability to inhibit a prepotent motoric response. During the task participants are required to rapidly respond to go signals. However on a percentage of trials the go signal is followed by a stop signal (e.g. auditory tone) requiring the participant to inhibit an already initiated response. As the go signal is reliably presented across all trials this produces a prepotency to respond. The delay between both signals varies across the task between 10 and 300 milliseconds. The need for longer latencies between the go and no-go signal in order to successfully inhibit a response, correlates significantly with the frequency of failures to withhold responding on the task and therefore is associated with poor inhibitory control (Logan et al., 1997). Longer reaction times to inhibit responses are argued to be indicative of a weakened inhibitory system.

The SST has more recently been replicated in animals subjects with some success (e.g. Feola, Richards and de Wit 2000; Eagle and Robbins, 2003). In the animal version of the SST animals, normally rodents, are required to respond to a visual go-signal. However, as with human task a no-go signal, an auditory tone, is randomly presented shortly following the go signal. Inhibition of the pre-potent behavioural response is then necessary and dependent on the version of the task requires the animal to either perform an alternative response (e.g. Feola et al., 2000) or withhold from responding further in order to be food rewarded (Eagle and Robbins 2003). As with human SST impulsivity is measured as the time required to inhibit responding, with longer RTs deemed more impulsive.

A third task used with human participants is the continuous performance task (CPT). Although arguably more a model of sustained attention, the task additionally assesses impulsive versus controlled motor behaviour. The test requires participants to respond as rapidly as possible to a

series of specified targets that are presented both briefly (less than 500msec duration) and rapidly (e.g. Rosvold et al., 1956; Dougherty et al., 1999). Impulsive responding is measured once again as the failure to suppress responding to non-specified targets, referred to as false alarms. A widely adopted task in the animal literature based on the human CPT is the five choice serial reaction time task (5CSRTT) (Carli, Robbins, Evenden and Everitt, 1983). This task, as with the CPT, is primarily used to assess sustained and divided attention in rodents. To be food rewarded, animals in the task are required to correctly respond, by nose poking, in one of five apertures that becomes illuminated. Impulsive behaviour can be assessed by the number of premature inappropriate responses that occur prior to the illumination of an aperture and commonly lead to a punished time out period (Robbins, 2002). The anticipatory response rate is paralleled to the false alarms made in the human CPT.

A final paradigm more commonly adopted with animal subjects to measure inhibitory responding is the differential reinforcement of low rate procedure (DRL) (e.g. van den Broek et al., 1987). In this operant model responses are only rewarded if the behaviour occurs following a fixed time interval. For example in a DRL 72", reinforcement is contingent on animals withholding a response for a minimum period of 72 seconds. Impulsive, premature responses are not rewarded and the fixed interval time is reset, usually following a time out period. The paradigm however, has been heavily criticised to be influenced by a number of factors other than inhibitory control, including motor performance (e.g. Evenden, 1998a) and timing ability (e.g. Paule, Meck and McMillan et al., 1999; Wiley, Compton and Golden, 2000). A variant of this procedure that attempts to dissociate the effects of drug induced activating or sedating effects on motor ability from impulsive responding is the fixed consecutive number procedure (FCN) (Mechner and Latranyi, 1963; Evenden, 1998a). Here the animal has to make a chain of responses on a lever before pressing a second lever to obtain a reward. Terminating the chain of responses before completion of the FCN results in no reward being delivered, and can be considered an impulsive act. More impulsive animals will perform fewer responses, switching to the second lever more prematurely.

1.3.2.2. Behavioural Tasks Measuring Impulsive Choice

Preference for smaller more immediate rewards over larger, more delayed rewards is argued to be a robust behavioural index of impulsivity (e.g. Rachlin and Green, 1972; Ainslie, 1974). In contrast 'self control' is regarded as the opposite preference where the ability to wait for the larger delayed reward is demonstrated. From this perspective impulsive behaviour is readily observed in drug abusers in that they frequently select the immediate acute reinforcing effects, or relief of drug withdrawal achieved from drug consumption over the long term larger social and health benefits associated with a drug free life style (Madden et al., 1997). One approach to the investigation and understanding of impulsive choices in drug dependence is the notion and

analysis of delayed discounting (DD) (Bickel and Marsh, 2001). Based on behavioural economics, DD refers to the fact that the subjective value of an outcome depreciates when it is delayed (Green, Fry and Myerson, 1994). Although the psychological assessment of DD originated from nonhuman-animal research (e.g. Chung and Herrnstein, 1967; Rachlin and Green, 1972; Ainslie, 1974), a number of procedures have been developed to assess delay aversion in human subjects.

Typically measures of DD are based on psychophysical choice procedures (e.g. Mazur, 1987; Richards et al., 1997). Tasks require human participants to make a choice between two rewards to enable the determination of the rate of discounting of the larger delayed reward over time. This can be achieved by either adjusting the magnitude or delay of rewards across the task to enable determination of the value of rewards at which reversal of reward preference takes place. In adjusted amount procedures for example (e.g. Richards et al., 1997; Mitchell, 1999; Reynolds, 2006b) participants are presented with two rewards, one of which is a larger, delayed reward (e.g. £1000 delivered in year), and the other, a smaller immediate reward the magnitude of which is adjusted until the preference of the immediate and delay rewards are approximately equal. This point of equal preference is referred to as the indifference point of that particular delay interval. During both tasks indifference points can be determined across a number of delays which can then be plotted to provide information of the rate in which the subjective value of the reward decreases with increasing delays of its delivery. Two models have dominated the literature that attempt to describe the affect of delay on discounting of rewards (eg. Ainslie, 1975; 1992). The exponential model, predicts that the subjective value of the delayed reward is discounted exponentially; that is for equal increments in delay of the production of the reward, there is a fixed proportion of decreased reward value (Kirby 1997) (1).

$$(1) \quad V = Ae^{-kD}$$

In the exponential function (1) V is the current subjective value of a delayed reward, (the indifference point), A is the value of the delayed reward, k is an empirically-derived constant proportional to the degree of delay discounting and D is the duration of delay of the reward. The larger the degree of discounting the more impulsive an individual is argued to be.

The second model instead argues that delayed reward is discounted hyperbolically; that is, devaluation of delayed rewards is proportional to their delay (Ainslie, 1992). For each unit of time that comprises the delay of delivery, the reward's current value decreases by an increasingly smaller quantity (2).

$$(2) \quad V = A/(1 + kD)$$

The majority of research comparing both models however has suggested that the hyperbolic function may be more effective at accounting for the discounting of delayed reward in both animal and humans subjects (e.g. Kirby, 1997; Richards et al., 1997; 1999a; Mazur, 2001; Frederick et al., 2003; Kirby and Santiesteban, 2003). More recently, in addition to the hyperbolic function researchers have begun to adopt area under the curve measurements as a further assessment of delay aversion (e.g. Myerson et al., 2001; Ohmura et al., 2005).

For most delay discounting laboratory procedures subjects make choices between ‘hypothetical’ outcomes, usually presented in the form of questions. Selection can be made for example of hypothetical monetary, drug or health outcome rewards (e.g. Madden et al., 1997; Odum et al., 2000; Chapman et al., 2001). The use of hypothetical outcomes enables the exploration of the impact of substantially larger rewards (e.g. £1000) and longer delays (e.g. one year) on impulsive choice which would be impossible to simulate with ‘real’ reward and delays. Furthermore, hypothetical rewards enable the ethical investigation of drug choices in human subjects (e.g. Petry and Bickel, 1998). In an attempt to both increase validity and reduce potential limitations associated with adopting hypothetical rewards, researchers in some instances have participants actually receive the outcome they preferred on one randomly selected choice trial from all choices made during the task (e.g. Kirby, 1997). More recently, tasks have been developed where both the rewards and delays are experienced, rewards however have been monetary and of considerably less value, with amounts for instance being less than £1 and delay to their delivery being no longer than an hour duration (e.g. Flora et al., 2003; Lane et al., 2003; Reynolds 2006a).

Animal models designed to assess impulsive choice as in human research are grounded in the temporal discounting theory of impulsivity. Although a number of variant delayed reward paradigms have been developed with the use of animals, in all models animals typically make a choice between a smaller reward and a delayed, larger reward. Thiebot et al. (1985) introduced this model using a maze-procedure, where at the end of one arm rats could consume two pellets immediately or at the end of the other arm animals were able to consume ten pellets only after the animal had been detained for a short period of time. The increased choice of the smaller immediate reinforcer is argued to be evidence of impulsivity. The model was later automated and delay of the reinforcer varied in order to achieve a delay function, which could be manipulated by drug treatment (e.g. Mazur, 1987; Evenden and Ryan, 1996; Richards et al., 1997). Such models can be divided into “systematic” and “adjusting” tasks. In the former animals, usually a rat, are trained to choose between one of two levers, one delivers a single food pellet immediately, the other delivers several pellets after a programmed delay. The delay to delivery of the larger reinforcer is under experimenter control and is increased systematically

during the testing session (e.g. Evenden and Ryan, 1996; Cardinal et al., 2000). Percent choice of delayed reward, across varying delays, is taken as index of impulsive choice. In contrast “adjusting” paradigms the magnitude of the immediate reward (Richards et al., 1997) or delay to the delivery of the larger reward (e.g. Mazur, 1987) is adjusted dependent on the animals choice behaviour, continuing across trials until the animal’s choice becomes indifferent between the two rewards. This point of indifference indexes the tolerance to delayed gratification, the smaller the magnitude of immediate reward or shorter the adjusted delay at this point the more impulsive the animal can be classified. Such models differ slightly from “systematic” delayed rewards measures, in that they measure the extent to which a subject values the standard alternative rather than which alternative reward is preferred.

Research that has adopted behavioural tasks in the attempt to define further the relationship between impulsivity and drug dependence can generally be categorised into those that have (i) compared levels of impulsivity in current substance abusers and non-substance abusers, (ii) compared amongst abusers’ levels of impulsivity across different pattern of substance misuse, (iii) compared levels of impulsivity in current users, ex-users and non-users, (iv) examined the acute and chronic effects of drugs of abuse on impulsivity, (v) examined the effects of acute drug deprivation on impulsivity and (vi) examined impulsivity as a predictor of drug abuse. Each of these categories will be reviewed incorporating findings from research that has focused upon both licit and illicit drug use.

1.3.2.3. Comparison of Levels of Impulsivity in Substance Abusers and Non-substance Abusers

A growing body of literature exists that suggest that substance abusers, of both licit and illicit drugs, display a greater sensitivity to delayed gratification in comparison to non-substance abusers. Madden and colleagues (1997; 1999) provided evidence of steeper discounting of hypothetical monetary rewards in opioid dependent individuals in comparison to matched controls. To reduce the subjective value of \$1000 by 60%, required a delay of one year for heroin abusers, in contrast to five years for the control group (Madden et al., 1997). Greater intolerance to delayed reward in heroin abusers was later replicated using a DD task, where participants were given the opportunity to win one of the reward choices selected during the task (Kirkby et al., 1999). In cocaine abusers comparable findings of heightened impulsive choice have in addition been found (Moeller et al., 2002; Coffey et al; 2003). A study conducted by Petry (2003) furthermore demonstrated that abusers of both cocaine and heroin discounted delayed gratification more rapidly than matched control regardless of the outcomes being presented. Steeper discounting was presented not only for hypothetical monetary rewards but also for commodities of health and freedom (although to a lesser extent). These findings may begin to explain the high risk behaviours shown by dependent users of illicit substances.

Abusers may engage in criminal behaviour to feed a drug habit or share needles to administer drugs due to the consequence of these behaviours possibly being delayed in time, and as a result being highly discounted (Petry, 2003).

Greater discounting of delayed reward has in addition been observed in abusers of licit drugs in comparison to control subjects, although arguably the findings have been less consistent. Whilst Petry (2001) found that alcoholics displayed a significantly greater intolerance to delayed monetary rewards in comparison to matched controls, these findings have failed to be replicated in a later study that compared the discounting rates of heroin, cocaine and alcohol abusers (Kirby and Petry 2004). Abusers of cocaine and heroin were found to display similar rates of discounting of delayed gratification that were significantly steeper than both alcoholics and matched controls, who displayed similar discounting rates. The lack of a significant difference between these two groups was argued to possibly be attributed to the self-selection bias of the participants, in that less impulsive individuals would more likely volunteer to take part in research and were therefore not representative of the general population of alcohol abusers (Kirby and Petry 2004).

Significant differences in DD have almost uniformly been found between smokers and non-smokers. Higher levels of impulsive choice of future monetary rewards have been demonstrated across a number of studies (e.g. Bickel et al., 1999; Mitchell 1999; Reynolds et al., 2004; Reynolds 2006a). Research has furthermore displayed a pervasiveness of the heightened impulsive choice in smokers. Higher levels of discounting in smokers have been found across differing reward magnitudes, during the consideration of both losses and gains (Baker et al., 2003), and when making choices of varying commodities including health outcomes (Odum et al., 2002). Two studies however have failed to demonstrate heightened impulsive choice in cigarette smokers. Firstly, no differences in discounting of monetary rewards were found between adolescent smokers between the ages of 14 and 16 and matched controls (Reynolds et al., 2003). Similarly, Ohmura and colleagues (2005) failed to demonstrate a difference between university students who smoked and non-smoking students. The average number of cigarettes smoked by participants across these studies varied, possibly accounting for the discrepancy in findings. In both of these studies participants were relatively “mild” smokers, with participants smoking an average 7 and 14 cigarettes a day in Baker et al., and Ohmura et al., studies respectively. By contrast, in those studies where highly significant differences were found, participants could be classified as “heavy” smokers, smoking no less than 20 cigarettes per day (e.g. Bickel et al., 1999; Mitchell 1999; Reynolds et al., 2004; Reynolds 2006a). These findings suggest that perhaps only individuals who are more dependent on tobacco display heightened sensitivity to delayed gratification.

Sensitivity to delayed reward has furthermore been examined in substance abusers with co-existing psychopathology. Comparable to findings adopting self report trait measures of impulsivity, evidence has been shown that substance abuse may interact with such disorders to have additive effect on intolerance to delayed reward. Petry and co-workers have demonstrated this association in problem gamblers with and without symptoms of substance abuse. Non-substance abusing pathological gamblers discounted more greatly delayed hypothetical rewards than matched controls (Petry, 2001b), and the substance abusing gamblers to an even greater degree than non-substance abusing gamblers (Petry and Casarella, 1999; Petry, 2001b). A comparable additive association has been demonstrated with substance abusers co-morbid with APD (Petry, 2002). Substance abusers, including those of cocaine, heroin and alcohol, additionally diagnosed with APD discounted delayed monetary rewards at a more rapid rate than substance abusers without APD. Both groups of substance abusers displayed more extreme impulsive choice relative to control subjects.

A further robust finding of empirical research is the significantly more rapid DD during choices involving the drug of dependence in comparison to other reward commodities. Hypothetical drug rewards have been found to be discounted to a much greater extent than monetary rewards across drug abusers including smokers (Bickel et al., 1999; Baker et al., 2003; Johnson et al., 2007), alcoholics (Petry 2001), cocaine (Coffey et al., 2003) and heroin abusers (Madden et al., 1997; 1999). To control for the effect of reward magnitude on delay discounting, the monetary value of the drugs were equated to the amount of the money utilised in the DD tasks.

It has been suggested that the negative reinforcement properties of drugs may account for the steeper discounting. Drugs are abused partly to relieve the aversive symptoms of withdrawal. For addicted individuals avoiding of these unpleasant symptoms may lead them to discount the delayed drug of abuse to a greater extent as such outcome would offer no immediate relief of withdrawal or prevention of its onset (Madden et al., 1997; Bickel et al., 1999; Petry 2001). Indeed, indirect evidence demonstrates that humans will make more impulsive choices when the reward allows them to avoid aversive consequences in comparison to when these consequences are pleasant (eg. Solnick et al., 1980). This explanation however cannot account for the steeper rates of discounting also found in both light smokers (Johnson et al., 2007) and social drinkers (Petry 2001) who arguably do not experience severe withdrawal symptoms. Increased discounting of drug rewards in comparison to monetary rewards could instead be explained by the fact that drugs are primary consumable reinforcers which tend to be discounted to a greater extent than conditioned, non-consumable reinforcers such as money. Odum and Rainaud (2003) demonstrated in non-addicted participants that both food and alcohol (primary, consumable reinforcers) were discounted similarly but to a much greater degree than monetary outcomes. Comparable findings were demonstrated in college students who were shown to discount pizza

more highly than money (Kirby and Guastello 2001). Related to this mechanism is the possibility that unlike money, drugs are perceived as a perishable commodity, that for example can stale over time, thus reducing more greatly their future value. Regardless of the mechanism involved, the discounting of the drug of abuse is extreme relative to other outcomes and is a common behavioural process observed across users of both licit and illicit drugs.

A potential criticism of the majority of the studies discussed is that subjects make choices regarding hypothetical rewards. Participants do not experience the delays associated with their reward choice as the outcomes are never delivered. There is however, growing evidence to suggest that discounting of both actual and hypothetical rewards are quantitatively similar. Across studies, hyperbolic functions have both consistently and accurately accounted for the discounting data of hypothetical rewards. Comparable hyperbolic functions have been furthermore displayed in studies adopting actual outcomes in both humans and animals (Mazur 1987, Richards et al., 1997). Although an earlier review of the discounting literature suggested that using hypothetical rewards may provide an underestimation of discounting rates (Kirby 1997), research has since concluded that within subjects there are no significant differences in the discounting rates of real and hypothetical rewards (Johnson and Bickel 2002; Madden et al., 2003; 2004; Lagorio and Madden 2005; Johnson, Bickel and Baker, 2007). Hypothetical rewards can therefore offer a valid methodology to assess levels of DD.

A significant amount of research has furthermore assessed levels of behavioural inhibition in individuals diagnosed with substance use disorders. Inhibitory control has been most widely studied in cocaine abusers in comparison to matched controls. Chronic cocaine abusers have consistently displayed a lower probability of successfully inhibiting responses on the SST and furthermore required significantly longer than matched controls to inhibit prepotent responses (Fillmore and Rush, 2002; Li et al., 2006). Compared to control subjects active cocaine abusers have in addition demonstrated evidence of a dysfunction in inhibitory control on the go/no-go task. Whilst no differences between groups were observed in the speed at which responses were executed, cocaine abusers made a significantly greater number of commission errors on the task, an impairment that has been found to be augmented with increasing demands on working memory (Kaufman et al., 2003; Hester and Garaven 2004).

Comparable inhibitory deficits have also been shown in opiate and methamphetamine dependent individuals, with poorer performance displayed on the go/no-go and SST task relative to demographically matched controls respectively (Forman et al., 2004; Monterosso et al., 2005). In contrast, no significant impairments in behavioural inhibition have been reliably shown in heavy ecstasy users utilising the go/no-go task (Fox et al., 2002; Quendow et al.,

2007). This suggests perhaps that abusers of this particular illicit drug may display minimal disruption in this particular component of impulsivity.

Research that has assessed differences in inhibitory control between current abusers of licit drugs and control non-abusing subjects has been limited. In smokers Spinella (2002) demonstrated that smoker status was significantly related to the number inhibitory errors on the go/no-go task, with smokers displaying a greater frequency of errors in comparison to non-smokers. No significant differences in performance on the go/no-go task were however found between college student smokers and non-smokers, despite smokers scoring significantly higher on the BIS non-planning sub-scale (Dinn, Aycicegi and Harris, 2004). As with the investigation of sensitivity to delayed reward in smokers, the relatively mild abusers in Dinn et al. sample, consuming on average 5.8 cigarettes per day, may account for the lack of significant relationship between smoking and impulsivity found in this study. Indeed a later study conducted by Yakir and colleagues (2007) using heavier female smokers, the majority of which smoked between 11-20 cigarettes per day, did provide evidence of significantly poorer inhibitory control on the CPT in comparison to matched controls.

In summary, the majority of research reviewed has demonstrated robust group differences between active abusers and non-abusers in both discounting of delayed reward and behavioural inhibition. Evidence has been displayed that both components of impulsivity are heightened in currently dependent individuals across illicit drugs of abuse, with the exception of ecstasy. In terms of licit drug abusers, a limited number of studies have assessed levels of impulsivity in currently abusing alcoholics and those that have been conducted have provided a discrepancy in findings. Clearly more research is needed to determine the relationship between impulsivity and active abusers of alcohol. In contrast, smokers have displayed significant evidence of both heightened impulsive choice and a dysfunction inhibitory control, an effect however perhaps limited to more heavy smokers. Despite evidence of the clear association between both components of impulsivity and addiction, once again the cross sectional design of the above studies does not enable the determination of whether heightened impulsivity is a consequence of the chronic abuse of these drugs, or contributes to the establishment and maintenance of addiction or both.

1.3.2.4. Comparison of Levels of Impulsivity Across Different Patterns of Substance Misuse Amongst Abusers

A limited number of studies have additionally assessed the relationship between impulsivity and the level and duration of drug abuse. The majority of research has focused upon different patterns of drinking and smoking behaviour. Vuchnich and Simpson (1998) for example compared the rates of discounting between light and heavy drinkers and light and problem

drinkers in college students. Findings demonstrated that in comparison to light drinkers higher levels of impulsive choice were shown in both heavy and problem drinkers. The greatest rate of delay discounting was observed in the most severe drinkers providing support for a dose dependent type relationship between drinking behaviour and impulsive choice. Field et al. (2007) have since replicated these findings in adolescent light and heavy drinkers where a more pronounced discounting of delayed reward was shown in participants with higher levels of alcohol consumption.

Within alcohol dependent individuals differences have in addition been found. Individuals diagnosed with early onset alcoholism were shown to display greater levels of discounting of delayed hypothetical rewards in comparison to late onset alcoholics, who failed to differ from matched controls in levels of impulsive choice (Dom et al., 2006). The lack of significant correlation displayed between years of abuse or dependence and rate of discounting led to authors concluding that findings suggested that heightened impulsive choice may be a risk factor in the development of early onset alcoholism.

Whilst no comparable studies have compared different patterns of alcohol abuse on measures of disinhibition, using a variant of the go/no-go task significant positive relationships between the severity of alcoholism and the inability to inhibit inappropriate responding have been reported (Noel et al., 2007). In a further study Bolla et al., (2000) found a significant relationship between the number of errors of commission on the go/no-go task and self reported level of alcohol use, findings consistent with a dose dependent type relationship between inhibitory control and alcohol consumption.

Less consistent findings have been displayed between social smokers, also referred to as 'chippers' and heavy smokers. Chippers are individuals who smoke cigarettes on a regular basis, normally within social environments, yet do not become addicted to tobacco. In a study where participants experienced both real rewards and delay to their delivery, individuals whom smoked more than 40 cigarettes per week displayed significantly higher rates of discounting of monetary rewards in comparison to both matched control non-smokers and lighter smokers who consumed in contrast less than 40 per week (Hyman and Gibb, 2006). The lighter smokers failed to differ from controls. In contrast, the lighter smokers in a study conducted by Johnson et al., (2007), failed to differ from heavy smokers with both smoker groups displaying significantly higher levels of impulsive choice of monetary rewards in comparison to never smokers. Furthermore, discounting of delayed reward was qualitatively similar across smoking groups, both in terms of magnitude and sign. Lower magnitudes of delayed monetary rewards were discounted to greater extent and discounting of both delayed health and monetary rewards were discounted greater when presented as gains rather than losses. The inconsistency in results

once again may be attributed to the varying classification of what is considered a light smoker. Smokers in Johnson et al., (2007) were considered light abusers if they smoked less than 10 cigarettes per day. This gave them opportunity to smoke more 70 per week, which is considerably higher than the classification for light smokers in Hyman and Gibb's study.

The absence of heightened impulsiveness in chipper smokers has in addition been replicated comparing heavy and occasional smokers on a possible measure of disinhibition, the CPT (Yakir et al., 2007). Heavy smokers in the study made significantly more errors of commission than both control and chippers, who reported smoking on average less than 10 cigarettes per week. Moreover, chippers actually displayed a significantly greater ability to withhold prepotent responses than the control participants (Yakir et al., 2007). As the CPT is a paradigm that primarily assesses attention, the positive effects observed on performance in light smokers may reflect more the enhancing attentional and vigilance properties of nicotine that have been established at lower more infrequent doses of the stimulant (e.g. Fan et al., 2002; Hahn and Stolerman, 2002). As previously discussed however, support for the lack of association between light smoking and disinhibition has been shown within a model principally utilised to measure inhibitory control, the go/no-go task. Adopting this task Dinn and colleagues (2004) failed to find a difference in performance between non-smokers and relatively mild smokers.

Further evidence that impulsivity not only discriminates between smoker and non-smokers but also within smokers, is demonstrated by the significant positive correlations reported across studies between self reported cigarette consumption and level of both discounting of delayed reward and frequency of commission errors (e.g. Spinella et al., 2002; Reynolds 2004; Reynolds et al., 2004; Ohmura et al., 2005; Reynolds, 2006a; Johnson et al., 2007).

Taken together, these data suggest that occasional, or light smokers and drinkers display little evidence of heightened impulsivity. This may be due to the lower levels of alcohol or nicotine these individuals have been exposed to relative to heavier abusers. Conversely, lower levels of impulsiveness prior to the initiation of smoking or drinking may protect these individuals from progressing from occasional use to more regular heavier use and dependence. The research conducted to date does not allow the direction of the relationship to be determined.

1.3.2.5. Comparison of Levels of Impulsivity in Current Users, Ex-Users and Non-Users

A number of studies have explored levels of impulsivity in individuals that are currently abstinent from drug use. Three studies, for example, have compared current substance abusers to former ex-users of the drug on tolerance to delayed gratification. Bickel and colleagues (1999) showed strong evidence of a reduction in sensitivity to the value of delayed rewards following the termination of smoking. In their study, smokers displayed significantly greater

sensitivity to delayed reward in comparison to not only non-smokers but also ex-smokers whom displayed similar discounting rates to controls. These findings suggest that the increased degree of discounting of rewards in smokers may be a reversible effect of nicotine dependence (Bickel and Marsch 2001). In contrast, further studies have found that former users display discounting rates intermediary between active users and controls. This has been observed when comparing alcoholics and currently abstinent alcoholics (Petry 2001) and active heroin/amphetamine injectors relative to former users (Bretteville-Jensen 1999). While these findings also suggest that drugs of abuse may result in an increase in impulsivity, an effect which is reduced with abstinence, they differ from that of Bickel et al., in that ex-users continue to differ significantly from never users. There are two plausible explanations for the discrepancy. Firstly, both former and current smokers in comparison to other drugs of abuse may simply display lower rates of discounting. Secondly, variations in the length of abstinence in former users across studies may account for the differences. In Bickel et al., study ex smokers had to have remained successfully abstinent for a least 12 months. Although Bretteville-Jensen did not report length of abstinence, former alcohol abusers had to have abstained from drinking for only a 1 month period. It could be that a critical period of abstinence is required before changes in DD reach comparable levels of non-users.

It is important to highlight here that a further interpretation across these studies is highly plausible. The fact that all former abusers did display lower levels of discounting of delayed reward than current abusers of the drug, could have resulted instead from a selection bias. A trait characteristic of low levels of impulsive choice may have allowed these individuals to successful abstain from the drug of abuse. Further research is essential to determine the direction of effect of low levels of impulsive choice and drug abstinence.

If it is the case, however, that with increasing length of abstinence a reduction in impulsive choice is observed, then according to this theory individuals that have abstained for a lesser period than the participants in the above studies should display levels of DD more comparable to that of current users. Indeed following 14 days of no drug use both abstinent cocaine and alcohol abusers were found not to differ in their degree of discounting relative to active users of each of these drugs (Kirby and Petry, 2004). Conversely, active and abstinent heroin abusers in the same study were found to differ, with lower levels of discounting shown by the ex users that were more comparable to the control group. The greater time that abstinent heroin abusers had been in treatment in comparison to ex cocaine and alcohol abusers, was argued possibly to have led to the differences found across abusers (Kirby and Petry 2004). Support for heightened impulsivity during early stages of abstinence has further been shown in methamphetamine users, who following one week abstinence continued to display significantly more extreme levels of discounting than matched controls (Hoffman et al., 2006). If it is to be argued that with

longer abstinence these participants will display greater tolerance to delayed reward, it does however have to be assumed that these individuals will continue to successfully remain abstinent. Due to the cross sectional design of the above studies no firm conclusions can therefore be made. Research is necessary that assesses, within subjects, impulsive choice from the point of termination of drug consumption through to long term abstinence (Mitchell, 2004; Reynolds, 2006b).

Whilst evidence has shown less extreme levels of discounting with long term abstinence for ex smokers (Bickel et al., 1999), alcoholics (Petry, 2001), heroin and amphetamine users (Bretteville-Jensen, 1999) comparable findings have not yet been indicated for ex cocaine users. Heil et al. (2006) demonstrated that no differences in sensitivity to delayed gratification were observed between active and ex users of cocaine who had been abstinent for a 30 day period. Furthermore, former cocaine users displayed significantly higher levels of impulsive choice relative ex users of heroin who had abstained from drug use for a comparable time period (Bornovalova et al., 2005). These findings suggest that, in contrast to other drugs of abuse, cocaine may not display a lesser reduction in impulsive choice following termination. Two possible explanations can account for these findings; firstly, the neuroadaptations that modulate the decrease in rates of DD may take a significantly longer period following chronic cocaine exposure relative to other drugs of abuse, secondly, in contrast, these findings may suggest instead that heightened impulsive choice in cocaine abusers is a pre-existing abnormality rather than a consequence of drug abuse.

In regards to changes in levels of behavioural inhibition with abstinence, only one study within the literature has directly compared both active and abstinent abusers on this aspect of impulsivity. Yakir et al., (2007) compared the performance of current smokers and ex-smokers who had abstained from smoking for at least six months on the CPT. Current smokers performed poorer on CPT than matched controls, making a significantly greater number of errors of commission. However, the greater impairment in the ability to inhibit pre-potent responses in smokers failed to differ from past smokers, who performed at an intermediate level, between active smokers and controls on the task. The comparable levels of heightened impulsivity in ex-smokers led to the authors concluding that evidence had been provided that impaired inhibitory control may be a vulnerability that can influence the initiation of smoking (Yakir et al., 2007). As with delay discounting research, no firm conclusions can however be made until further longitudinal studies determine whether with continued abstinence ex-smokers continue to improve in their ability to inhibit behaviour. As shown in the case of impulsive choice, following a longer period of abstinence (one year) ex smokers did display significantly lower levels of impulsivity in comparison to current smokers (Bickel et al., 1999).

Whilst limited studies have been conducted comparing disinhibited behaviour of current and ex users, more extensive research has compared populations of abstinent users to control subjects. Following 15 days of abstinence, cocaine but not heroin dependent individuals, displayed a greater dysfunction in the ability to inhibit inappropriate responding on a version of the go/no-go task in comparison to matched control participants (Verdejo-Garcia, Perales and Peres-Garcia 2007). The more extreme levels of impulsivity demonstrated by abstinent cocaine users compare to findings exploring sensitivity to delayed gratification, where cocaine but not heroin dependent individuals once again demonstrated significant differences relative to controls (Kirby and Petry, 2004). The greater neurological impairment in chronic cocaine abusers has been argued to possibly underlie variations in performance between abusers (Lyoo et al., 2004). Whether impairments in inhibitory control in cocaine abusers persist with longer abstinence however, is unclear.

Evidence of heightened impulsive control has furthermore been demonstrated in abstinent alcoholics in comparison to non-alcohol dependent individuals. Following a minimum abstinence period of two weeks, detoxified alcoholics continued to display greater deficits in response inhibition on a modified go/no-go task where both neutral and alcohol related words represented go target signals (Noel et al., 2005; 2007). Evidence of continued impairment with longer term abstinence has also been shown. Heightened disinhibition, as measured by commission errors, has been demonstrated in alcoholics at 28 days of abstinence on the CPT (Bjork et al., 2004) and 3 months following termination of drinking on the go/no-go task (Goudriaan et al., 2005). Taken together these findings suggest that in alcoholics dysfunctions of inhibitory control persist long after termination of consumption. Whether this impairment predated alcoholism or is instead a consequence of chronic alcohol exposure is unclear. It is important to acknowledge, however, that in many of the studies smoking behaviour was not controlled for. The loss of inhibitory control associated with smoking could therefore account for the continued heightened impulsivity observed in these subjects.

In summary, studies that have included current and past, abusers suggest that heightened impulsivity, in particular intolerance to delayed reward, may be a reversible effect of drug dependence. Furthermore, there may be a critical period of abstinence, that varies across drugs of abuse, before a reduction in DD can be shown. However, due to the cross sectional nature of the research a further plausible interpretation of findings is that lower levels of impulsivity has enabled successful abstinence. No firm conclusions can be made regarding the association between disinhibition and abstinence from drug abuse due to the sparse number of studies including both an active and abstinent drug group. However, with the exception of heroin abusers, studies that have compared users in both early and later stages of abstinence to non drug users have demonstrated consistent poorer levels of inhibitory control. This suggests the

possibility that unlike heightened impulsive choice, chronic drug exposure may lead to long term changes in inhibitory control, that persist following the termination of drug abuse. Conversely, these findings could indicate that impairments in behavioural control could have predated drug exposure and constituted instead a vulnerability for developing addiction. Only research that assesses levels of both components of impulsivity longitudinally prior to, during and following drug abuse will enable conclusively to determine the temporal association between impulsivity and drug dependence.

1.3.2.5. Examination of the Acute and Chronic Effects of Drugs of Abuse on Impulsivity

For many of the reviewed aforementioned studies it is unclear whether participants during the completion of tasks are under the acute influence of the abused drug or in a state of withdrawal and importantly to what extent these drug states may be affecting performance. In attempt to explore more directly the impact of drugs of abuse on both impulsive choice and disinhibition, a number of studies have examined the acute, and to a lesser extent chronic, drug effects in both human and animal models of impulsivity. Table 1.1-1.4 summarise the acute and chronic effects of drugs of abuse on impulsivity.

Repeated cocaine administration has been shown to increase preference for the immediate smaller reinforcer in a rodent model of DD (Logue et al., 1992). Comparably, Paine and colleagues (2003) later demonstrated evidence of heightened impulsive choice during a two week period of cocaine administration. Animals treated three times a day with 15mg/kg cocaine displayed reduced indifference points in comparison to saline treated animals in the first seven days of treatment. This effect however recovered and returned to baseline levels during the final seven days of treatment, explained by the authors as an increased tolerance to cocaine's effects on impulsive behaviour (Paine, Dringenberg and Olmstead, 2003). No comparable assessments of cocaine administration on DD have been studied in human subjects.

Acute cocaine has furthermore been studied in models of inhibitory control. In rodents acute cocaine has been found to increase the inability to withhold inappropriate responding as assessed in DRL (Cheng, MacDonald and Meck, 2006), asymmetrically reinforced go/no-go paradigm (Paine and Olmstead 2004) and the 5CSRTT (Van Gaalen, Brueggeman, Bronius et al., 2006). The induced increase in impulsive responding parallels the impaired inhibitory control found in chronic cocaine abusers on following oral consumption of low doses of cocaine hydrochloride on the SST (50-150mg/kg) (Fillmore et al., 2002).

More recently, however, within the human literature, reports by the same authors have been made of a decrease in impulsivity following cocaine consumption. In chronic cocaine abusers oral consumption of cocaine hydrochloride across doses of 100, 200, and 300mg/kg led to an

linear increased ability to inhibit responding in a cued dependent version of the go/no-go task (Fillmore, Rush and Hays, 2005; 2006a). Furthermore reaction time to inhibit responses was also found to be reduced in the SST, indicative of an increase in inhibitory control, under 100 and 200mg/kg but not the highest cocaine dose (Fillmore, Rush and Hays, 2006b). Authors suggest that perhaps a nonlinear, U-shaped affect of acute cocaine on impulsive behaviour may exist. Lower and possibly higher doses of cocaine may impair inhibitory control whilst intermediary doses may have a more positive effect on impulsivity (Fillmore et al., 2005). As already outlined chronic abusers of cocaine have consistently demonstrated heightened dysfunctions in inhibitory control (e.g. Hester and Garaven, 2004; Li et al., 2006). Drug users may initially abuse moderate doses of cocaine as a form of self medication, to perhaps aid in the control of disinhibited behavioural tendencies (e.g. Khantzian, 1985). With repeated cocaine exposure however, this may give rise to neuroadaptations that lead to greater impairments of inhibitory mechanisms, resulting in a loss of control over drug intake and sustaining of drug abuse (e.g. Fillmore et al., 2005). In support of this theory repeated cocaine treatment for a 14 day period in monkeys led to an increase in perseverative responding on an object discrimination task, indicative of disinhibited behaviour (Jentsh, Olausson, Garza et al., 2002). Evidence was furthermore provided that these impairments were still present 30 days following termination of treatment. The persistence of performance deficits following long term termination of treatment are comparable to the continued impaired inhibitory control observed in abstinent cocaine users (e.g. Verdejo-Garcia et al., 2007).

The effect of a further psychostimulant extensively studied on impulsive behaviour is that of amphetamine and its derivatives. In the animal literature acute doses of the stimulant drug, as well as its analogue methamphetamine, have yielded inconsistent results in models of sensitivity to delayed reward. Both a suppression (Richards et al., 1999a; Cardinal et al., 2000; Wade et al., 2000; Winstanley et al., 2003a; Van Gaalen et al., 2006) and enhancement of impulsive choice has been reported in rodents (Charrier and Thiebot, 1996; Evenden and Ryan, 1996; Cardinal et al., 2000; Isles, Humby and Wikinson 2003; Helms, Reeves and Mitchell 2006). The discrepancy in findings following acute administration may possibly be accounted for by procedural differences across studies. Studies that reported a significant increase in impulsive choice have utilised food reinforcements in systematic delayed reward paradigms. In contrast, water rewards were adopted in adjusted amount procedures that conversely demonstrated a reduction in impulsivity. The anorectic properties of the stimulant (e.g. McPhail and Gollub, 1974) may have resulted in varying effects on the motivation for food and water rewards leading to differential effects on the choice of reinforcement in each of the paradigms.

Furthermore, the differences in findings may be accounted for by the presence or absence of a reward-predicting cue during the delay to delivery of the larger reward. In studies where

amphetamine suppressed impulsive choice a reward-predicting cue (a tone, or light) was presented during the duration of delay to the delivery of the larger reward (Richards et al., 1997; 1999a; Wade et al., 2000). The cue-dependent effect of amphetamine to increase tolerance to delayed reward was supported later by Cardinal and colleagues who directly compared the acute effects of d-amphetamine on choice of both signalled and unsignalled delayed reward tasks (Cardinal et al., 2000). In support of previous research, amphetamine displayed differential effects dependent on the presence or absence of a cue, with a reduction and enhancement of impulsive choice demonstrated respectively. Authors argued that the signal presented in cued paradigms becomes a conditioned reinforcer to the delivery of the larger reward. Amphetamines ability to therefore promote choice of the delayed reward is through the stimulants potentiation of the conditioned reinforcing properties of this signal, thus leading to the enhanced selection of the associated reward (Cardinal et al., 2000).

More recently, Isles and colleagues (2003) have indicated that the promotion of impulsive choice or increase in self control by d-amphetamine may be dependent on the dose administered. A biphasic response to the psychostimulant was evident in mice. Indication of a decrease in impulsivity was observed at the lower dose range in contrast to enhanced impulsive responding at the higher doses tested. Support for the theory that low doses of amphetamine might promote tolerance of delayed reward has been provided by the single study that has been conducted with human subjects. In healthy subjects a low 20mg dose of d-amphetamine increased preference for the larger more delayed reward (de Wit et al., 2002). These findings suggest that perhaps low to moderate, infrequent doses of d-amphetamine may reflect the known therapeutic effects of the stimulant on impulse control in ADHD diagnosed patients (e.g. Tannock et al., 1989). In contrast higher doses, more comparable to those administered in methamphetamine dependent individuals, may lead to heightened levels of impulsive choice. Indeed Richards et al. (1999a) demonstrated a significant increase in impulsive choice following chronic 14 day administration of high doses (4.0mg/kg) of methamphetamine in rats, a treatment regime more comparable to the pattern of drug use observed in dependent individuals.

The dose dependent effect of acute amphetamine treatment has additionally been indicated in models of inhibitory control. Consistent to the drug effects on increasing tolerance to delayed reward low to moderate doses of the stimulant have been shown to facilitate inhibitory control. In healthy participants the stimulant drug (10 and 20mg/kg), has been shown to both selectively decrease frequency of inhibitory failures on the go/no-go task and to reduce time taken to inhibit a response on the SST (de Wit et al., 2000; 2002). The improvement in inhibitory control however is restricted to those individuals displaying a poor baseline level of inhibitory control (de Wit et al., 2000; 2002; Fillmore et al., 2005). The baseline rate dependent effect is consistent

once again with the therapeutic properties of the drug in individuals with already poor inhibitory control (e.g. Tannock et al., 1989).

Parallel to these findings, adopting a rat version on the SST low doses of 0.25 and 0.5mg/kg of d-amphetamine improved inhibitory control, an effect restricted to animals exhibiting a low baseline level of performance (Feola, de Wit and Richards, 2000). Higher doses tested in animal models have in contrast demonstrated evidence of the drugs ability to induce impulsive responding. At doses across the range of 0.8-2.4g/kg, a reduction in inter response time in the DRL (Wiley, Compton and Golden, 2000) as well as an increase in extreme premature responding in FCN (Evenden 1998a; 1998b) have been shown. Furthermore, following acute amphetamine both impairments in performance on an asymmetrically reinforced go/no-go task (Ridley et al., 1980) and enhanced anticipatory responding in the 5CSRTT have been reported (Harrison et al., 1997; Van Gaalen et al., 2006).

State changes in impulsivity have in addition been examined following consumption and administration of licit substances. Firstly, the assessment of alcohol intoxication has revealed no changes in sensitivity to delayed reward in social drinkers following the consumption of alcohol (Richards et al., 1999b; Reynolds et al., 2006b), although one study did report evidence of perhaps a slight decrease in levels of impulsive choice (Ortner et al., 2003). It has been argued however that DD measures where delay to the delivery of reward is not experienced may lack sensitivity to the acute effects of alcohol (McDonald et al. 2003; Reynolds and Schiffbauer, 2004). This was supported by a later study by Reynolds and colleagues (2006b) who adopted a DD measure, the Experiential Discounting Task (EDT), where both reward and delay to its delivery were experienced by the participant. The highest 0.8g/kg alcohol dose tested was found to significantly increase impulsive choice relative to the placebo condition. Comparably, in animal models of delayed reward, where subjects also receive selected rewards and are exposed to delays, acute ethanol has led to a significant increase in preference for the smaller more immediate reward across DD models in rats (Tomie et al., 1998; Evenden and Ryan, 1999; Hellmans, Nobrega and Olmstead, 2005).

Explored more extensively are the effects of alcohol intoxication on the ability to inhibit inappropriate responding as a measure of impulsivity. A selective increase in inhibitory failure on the SST has been observed following both moderate doses of alcohol in social drinkers (e.g. Mulvihill et al., 1997; Fillmore and Vogel-Sprott, 1999; De Wit, Crean and Richards et al., 2000; Easdon and Vogel-Sprott 2000; Reynolds et al., 2006b) and ethanol in rats (Feola et al., 2000). Comparable increases in impulsivity moreover have been demonstrated in a more challenging version of the CPT, the immediate and delayed memory tasks. Both moderate (0.20g/kg) and higher doses (1.0g/kg) of alcohol increased frequency of commission errors,

although at the higher dose a decrease in attention was also shown which could have accounted for the poorer performance on the task (Dougherty et al., 1999; 2000). Evenden (1998a; 1998b) in rats further demonstrated decrease in chain length of responses in an FCN procedure at doses 1.0 and 3.0mg/kg, although once again, effects have not always been selective with a reduction in total responding also observed at the highest dose (Evenden, 1998b).

In contrast, less consistent findings have been found with acute effects of alcohol on the go/no-go task. Reports of both an increase in inhibitory failure (Finn et al., 1999; Easdon et al., 2005) and a lack of an effect of alcohol on performance have been made (Ortner et al., 2003; Reynolds et al., 2006b). The lack of consistency of the effects of alcohol in the go/no-go task relative to the SST has led authors to argue that both tasks may differ in their underlying processes (e.g. Reynolds et al., 2006b). Unlike the SST, where an already initiated response has to be retracted, reflecting perhaps more motor impulsivity, the go/no-go task requires instead participants to both remember go-signal targets and to decide whether or not to respond. The latter task therefore is arguably a more cognitively demanding paradigm. When not under the influence of drugs, however, performance on both tasks have been found to be positively related, thus supporting common underlying processes of both behavioural inhibition tasks (Reynolds et al., 2006a). Interestingly, in cue dependent versions of the go/no-go paradigm alcohol has reliably increased disinhibition. In such paradigms prior to Go and No-go signals cues are presented that either have an 80% reliability of either a presentation of Go and No-go signal then following (e.g. Marcinski and Fillmore, 2003a; Marcinski and Fillmore, 2003b; Fillmore and Weafer, 2004; Marcinski and Fillmore, 2005; Marcinski et al., 2005). Alcohol has been found to dose dependently increase failures to inhibit responses only during trials where incorrect Go but not correct No-go cues are presented, prior to the signal. These findings suggest that under drug influence individuals may become more reliant on cues to inhibit behaviour, and that impairments in inhibitory behaviour are most likely to occur when there is a particular strong responding tendency.

The effects of nicotine have also been explored. Only one study has however assessed the effects of nicotine in a model of DD. In rats Dallery and Locey (2005) demonstrated that across the acute dose range tested (0.03-1.0mg/kg), a dose dependent increase in intolerance to the delayed larger reward was shown. In the same study, animals then received 0.3mg/kg of nicotine for a duration of 65 days, following which the acute dose regime was then repeated. Impulsive choice was substantially heightened following all doses, including saline, providing evidence of greater levels of discounting during chronic exposure. Interestingly, following the termination of chronic treatment animals remained more impulsive for approximately four weeks before a return to baseline levels was shown. These findings compare to that of Bickel et al. (1999), that displayed differences in impulsive choice between human smokers and ex-

smokers, supporting the interpretation that nicotine dependence may lead to reversible increases in impulsivity.

Nicotine has more frequently been examined in models of inhibitory control. Findings are complex however, with mixed results reported. In a group of healthy non-smokers administration of nicotine for 4.5 hours via a transdermal patch had little effect on inhibitory control whilst significantly improving sustained attention on a CPT task (Levin et al., 1998). Comparably, in moderate smokers acute nicotine (7 and 21mg) had no effects on impulsive responding following overnight abstinence, as assessed by both the CPT and SST (Bekker et al., 2005). As with amphetamine, evidence has however been demonstrated of positive effects of the stimulant on inhibitory control in populations associated with disinhibited behaviour at baseline. For example, in non-smoking adolescents diagnosed with ADHD acute nicotine (7mg/day) was found to significantly increase inhibitory control as measured by the decrease in reaction time taken to inhibit responses on the SST (Potter and Newhouse, 2004). Schizophrenia is a further psychopathology associated with heightened impulsivity (e.g. Dervaux et al., 2004), where high doses (21mg) of nicotine have been shown to markedly reduce commission errors on the CPT in patients (Levin et al., 1996). In the same group of patients under the influence of lower dose of nicotine (7mg) impulsive responding was however enhanced, with an increase in commission errors shown (Levin et al., 1996). The potential therapeutic effects of nicotine on inhibitory control in such patient groups is believed to be associated with the high prevalence of smoking observed in these populations (de Leon et al., 1995; Pomerleau et al., 1995; Dervaux et al., 2004; Gehricke et al., 2006). If this is indeed the case, then heightened impulsivity, at least in these patient groups, may predispose these individuals more vulnerable to the development of nicotine addiction.

Within the animal literature nicotine has in addition yielded mixed results. The drug has been most extensively been examined in the 5CSRTT. Although the main focus of such research was the exploration of acute nicotine's enhancing properties on attention, the stimulant has demonstrated heightened anticipatory responding following its administration in both mice and rats (e.g. Mirza and Stoleran, 1998; Blondel, Sanger and Moser, 2000; Stoleran, Mirza, Hahn, Shoaib, 2000; Hahn, Shoaib and Stoleran, 2002; Bizarro, Patel, Murtagh, Stoleran, 2004; Bruin, Fransen, Duytschaever, Grantham and Megens, 2006). The increased inability to withhold inappropriate responding was generally observed following low doses of nicotine (0.03-0.3mg/kg) and under conditions of high attentional demand. Other research however has failed to demonstrate an effect on anticipatory responding in nicotine naïve animals, yet have reliably displayed an increase in impulsive responding in animals previously treated with nicotine (Grottick and Higgins 2000; 2001; Day, Pan and Buckley, et al., 2007). Comparably, in a further study where the effects of repeated nicotine exposure were assessed over a seven day

period, no effect on anticipatory responding was observed during initial treatment. With continued exposure however a more impulsive behavioural profile then predominated (Blondel, Simon, Sanger and Moser, 1999). Caution must however be given in interpreting these findings as evidence of heightened impulsivity. The increase in anticipatory responding could potentially reflect nicotine induced hyperactivity, an effect well established in animals chronically exposed to nicotine (e.g. Clarke and Kumar, 1983). Particularly in studies where combined decreases in response latency are exhibited, it becomes increasingly difficult to dissociate nicotine's effects on sensitisation of motor functions from that of inhibitory control (Blondel et al., 1999; Grottick and Higgins 2000; 2001).

Further support for a nicotine induced reduction in inhibitory control has been demonstrated following its administration in the DRL paradigm. An increased inability to withhold responding indicated by a greater response rate and premature responding has reliably been shown across studies (Morrison, 1968; Bizot 1998; Popke, Mayorga, Fogle and Paule 2000a; 2000b). The DRL model is however additionally sensitive to both changes in motor, and timing ability, both factors known to be affected by nicotine (e.g. Clarke and Kumar, 1983; Carrasco et al., 1998). It is therefore difficult to validly interpret these findings as evidence of nicotine induced loss of inhibitory control.

Overall the effects of nicotine on inhibitory control appear to be complex and dependent on a number of factors including dose, length of exposure and baseline level of impulsivity. The reported positive effects of nicotine in the human literature on inhibitory control appear seemingly in contrast to the poor impulse control evident in smokers (e.g. Spinella, 2002; Yakir et al., 2007). It should be noted however that these findings were demonstrated in individuals with diagnosed psychopathology, limited studies have been conducted exploring the effects of acute nicotine in both healthy smokers and non smokers therefore making it difficult for valid conclusions to be made. It may however be the case, unlike sensitivity to delayed reward which has shown to increase with nicotine treatment, that poor inhibitory control may be risk factor in the development of smoking. As argued with cocaine, individuals may initially smoke as a form of self medicating and controlling of disinhibited behaviour. However, with repeated nicotine exposure, this may lead to neuroadaptations that lead to greater dysfunctions in inhibitory control as supported by the chronic exposure to nicotine in animals (e.g. Blondel et al., 1999). Further research is necessary that explores the impact of acute and chronic nicotine in both healthy smokers and non-smokers, whilst the need for animal research to assess nicotine treatment in models explicitly designed to measure inhibitory control is essential.

The impact of additional drugs of abuse has also been examined in models of impulsivity, however to a much lesser extent. For example the opiate morphine has demonstrated a dose

dependent increase in discounting of delayed reward following its acute administration in rats, an effect blocked by the opioid antagonist naloxone (Kieres, Hausknecht, Farrar, et al., 2004). In models of behavioural inhibition acute treatment with the commonly abused drug, THC, has furthermore displayed evidence of increased levels of disinhibition, as indicated by the augmented impulsive responding on a SST in human subjects reporting previous use of the drug (McDonald et al., 2003), and enhanced levels of premature responding in a DRL task in rats (Wiley et al., 2000). Despite reported alterations in inhibitory control, no significant effects have however been reported on impulsive choice following the intake of THC (McDonald et al., 2003).

In summary, abused drugs have been shown to have distinctive effects on measures of impulsivity. Increasing evidence across drugs of abuse suggests that both administration and consumption may lead to an increased sensitivity to delayed gratification, these effects however may be restricted to both higher and chronic doses more comparable to the abuse of drugs in dependent individuals. The effects of drugs on inhibitory control appear to be less consistent and more complex. In the case of the stimulants, cocaine, amphetamine and nicotine their effects on inhibitory control appear to dependent on a number of factors including dose level, length of drug exposure and baseline impulsivity. Acute low to moderate doses have demonstrated positive enhancing effects of inhibitory control. These potential benefits are specific however to individuals whose basal levels of inhibitory control are sub-optimal. Although limited, evidence does suggest that more long term chronic exposure to high doses of such stimulants may lead to substantial deficits in inhibitory control, more consistent with the levels of impulsivity observed in drug dependent individuals. When considering the relationship between impulsivity and drug dependence, these findings, to some extent, suggest evidence that drugs of abuse may directly impact upon levels of impulsive behaviour, although the initial and long term effects may differ. It is essential that future research in both human and animals explore the effects of drug regimes more comparable to that adopted by drug dependent individuals. Such investigations will enable determination of the required length and amount of drug exposure necessary to elicit the neuroadaptations that may mediate the heightened impulsivity evident in drug dependent individuals.

Table 1.1: The Acute and Chronic Effects of Drugs of Abuse on Impulsive Choice in Human Participants

<u>Authors</u>	<u>Drug</u>	<u>Paradigm</u>	<u>Sample</u>	<u>Dose</u>	<u>Design</u>	<u>Analysis</u>	<u>Effect</u>	<u>Comments</u>
De Wit et al., 2002	D-AMPHETAMINE	Adjusting Amount DD Task. Hypothetical monetary rewards with opportunity to select at random one of the reward choices.	N = 36 Healthy male & female	Acute: 10 & 20mg oral capsules.	RM	1-way RM ANOVA	↓ (20mg)	
Richards et al., 1999b	ALCOHOL	Adjusting Amount DD Task. Hypothetical monetary rewards with opportunity to select at random one of the reward choices.	N = 24 Healthy male & females	Acute: 500 & 800mg/kg beverage	RM	RM t-tests	—	
Ortner et al., 2003		Adjusting Amount DD Task. Hypothetical monetary rewards with opportunity to select at random one of the reward choices	N = 76 Healthy males	Acute: 700mg/kg beverage	BS	2-way ANOVA	↓	

Reynolds et al., 2006b	EDT- All rewards and delay to its delivery were experienced by the subject. Adjusting Amount DD Task. Hypothetical monetary rewards with opportunity to select at random one of the reward choices	N= 24 Healthy male and female social drinkers	Acute: 400 & 800mg/kg beverage	RM	2-way RM ANOVA	— Adjusting Amount DD task ↑ (800mg) EDT	EDT tasks may be more sensitive to the acute effects of alcohol due to all rewards and delay to their delivery is experienced.
Mc Donald et al., 2003	Adjusting Amount DD Task. Hypothetical monetary rewards with opportunity to select at random one of the reward choices	N= 37 Healthy male and female	Acute: 7.5 & 15mg oral capsule	RM	2-way RM ANOVA	—	
THC							

↑ Indicate that the drug increased impulsive choice significantly in comparison to control treatment p< 0.05. ↓ Indicate that the drug decreased impulsive choice significantly in comparison to control treatment p< 0.05. — Indicate that drug did not display any behavioural effects on impulsive choice.

Abbreviations: ANOVA , Analysis of Variance; DD, Delay Discounting; BS, Between Subjects; EDT Experimental Discounting Task; RM, Repeated Measures; THC, Tetrahydrocannabinol

Table 1.2: The Acute and Chronic Effects of Drugs of Abuse on Impulsive Choice in Animal Subjects

<u>Authors</u>	<u>Drug</u>	<u>Paradigm</u>	<u>Sample</u>	<u>Dose</u>	<u>Design</u>	<u>Analysis</u>	<u>Effect</u>	<u>Comments</u>
Logue et al., 1992	COCAINE	Adjusting DD Task	N = 5 male LH rats	Subchronic: 15mg/kg i.p. immediately prior to session	RM	RM t-test	↑	
Paine et al., 2004		Systematic DD Task	N = 16 male LE rats	Chronic: treated with saline or 15mg/kg i.p. three times per day for a 14 day period	MD	3-way RM ANOVA	↑ for first 7 days	Possible development of tolerance to cocaine's effects observed during final 7 days of treatment.
Charrier & Thiebot, 1996	D-AMPHETAMINE (METHAMPHETAMINE)	T-Maze Delayed Reward Task	N = 16 male W rats	Acute: 0.25, 0.5 1.0mg/kg i.p.	RM	1-way RM ANOVA	↑ (0.5mg/kg)	
Evenden & Ryan, 1996		Systematic DD Task	N = 24 male SD rats	Acute: 0.3 & 1.0mg/kg i.p.	RM	2-way RM ANOVA	↑ (1.0mg/kg)	
Richards et al., 1999a		Adjusting Amount DD Cued Task	N= 19 male SD rats	Acute: 0.5, 1.0, 2.0 & 4.0mg/kg methamphetamine i.p. Chronic: 4.0mg/kg methamphetamine for a 14 day period	RM	1-way RM ANOVA	↓ Acute (0.5, 1.0 & 2.0mg/kg) ↑ Chronic	
		Systematic						

Cardinal et al., 2000	DD Cue and No CueTask	N = 16 male LH rats (N = 8 Cue; N = 8 No Cue)	Acute: 0.3, 1.0 & 1.6mg/kg i.p.	RM	2-way RM ANOVA	↑ No Cue (1.0 & 1.6mg/kg) ↓ Cue (0.3mg/kg)	Effects of amphetamine appear to be cue-dependent. Cue presented during the delivery of delayed, larger reward may become a conditioned reinforcer. Amphetamine may potentiate conditioned reinforcing properties of the cue.
Wade et al., 2000	Adjusting Amount DD Cued Task	N = 17 male SD rats	Acute: 0.5 & 1.0 mg/kg i.p.	RM	1-way RM ANOVA	↓ (0.5 & 1.0mg/kg)	
Isles et al., 2003	Systematic DD Cued Task	N = 16 male C57B1/6Xcba/CA mice	Acute: 0.4, 0.6, 0.8 & 1.0 mg/kg i.p.	RM	2-way RM ANOVA	↓ (0.4 & 0.6mg/kg) ↑ (0.8mg/kg)	Effect of amphetamine on impulsive choice appears to be dose dependent. A biphasic response was evident.
Winstanley et al., 2003a	Systematic DD No Cue Task	N = 10 male LH rats	Acute: 0.3, 1.0 & 1.5, 2.3 mg/kg i.p.	RM	2-way RM ANOVA	↓ (0.3, 1.0 & 1.5, 2.3mg/kg)	
Van Gaalen et al., 2006	Systematic DD No Cue Task	N = 16 male W rats	Acute: 0.2, 0.5 & 1.0mg/kg	RM	2-way RM ANOVA	↑ (0.2, 0.5 & 1.0mg/kg)	
Helms et al., 2006	Amount DD Task	N = 12 C57BL/6J mice N = 12 DBA/2J mice	Acute: 0.4, 0.8 & 1.2mg/kg i.p.	RM	2-way RM ANOVA	↑ (0.8 & 1.2mg/kg)	

Kieres et al., 2004	MORPHINE	Adjusting Amount DD Task	N = 17 male rats	SD	Acute: 0.3, 1.0 & 1.8mg/kg s.c.	RM	1-way RM ANOVA	↑ (1.8mg/kg)
Tomie et al., 1998	ETHANOL	T-Maze Delayed Task	N = 15 male rats	LE	Acute: 250, 500, 1000, 1500mg/kg i.p.	RM	MANOVA	↑(250, 500 & 1000mg/kg)
Evenden & Ryan, 1999		Systematic DD Task	N = 16 male rats	SD	Acute: 0.3 & 1.0mg/kg s.c.	RM	1-way RM ANOVA	↑ (1.0mg/kg)
Hellmans et al., 2005		Systematic DD Task	N = 36 male rats	LE	Acute: 0.3, 0.6, 0.9, 1.2mg/kg i.p.	RM	RM ANOVA	↑ (Dose dependant effect 0.3, 0.6, 0.9 & 1.2mg/kg)
Dallery & Locey, 2005	NICOTINE	Adjusting Delay DD Task	N = 5 male rats	SD	Acute: 0.03, 0.1, 0.3 & 1.0mg/kg s.c. Chronic: 0.3mg/kg s.c. once per day for 65 days followed by acute dose regime	RM	2-way RM ANOVA	↑ Acute (0.1, 0.3 & 1.0mg/kg) ↑ Chronic (impulsive choice greater following administration of saline & 0.03mg/kg relative choice behavior following acute regime) Following termination of chronic treatment animals remained more impulsive for a 4 week period.

↑ Indicate that the drug increased impulsive choice significantly in comparison to control treatment $p < 0.05$. ↓ Indicate that the drug decreased impulsive choice significantly in comparison to control treatment $p < 0.05$. — Indicate that drug did not display any behavioural effects on impulsive choice.

Abbreviations: ANOVA, Analysis of Variance; DD, Delay Discounting; BS, Between Subjects; i.m. Intramuscular; i.p, intraperitoneal; LE, Long Evans; LH, Lister Hooded; M, Mixed Design; MANOVA Multivariate Analysis of Variance; RM, Repeated Measures; RT, Reaction time; THC, Tetrahydrocannabinol ; s.c., subcutaneous; SD, Sprague Dawley ; W, Wistar

Table 1.3: The Acute and Chronic Effects of Drugs of Abuse on Inhibitory Control in Human Participants

<u>Author</u>	<u>Drug</u>	<u>Paradigm</u>	<u>Sample</u>	<u>Dose</u>	<u>Design</u>	<u>Analysis</u>	<u>Effect</u>	<u>Comments</u>
Fillmore et al., 2002	COCAINE	SST	N = 8 male & females with a history of cocaine use	Acute: 50, 100 & 150mg oral capsule	RM	1-way RM ANOVA	↑ (100, 150mg)	Selective effect. Cocaine reduced ability to inhibit responses without affecting the participant's estimated reaction time to inhibit or execute responses.
Fillmore et al., 2005		Cue Dependent Go/No-go Task	N = 14 male & females with a history of cocaine use	Acute: 100, 200 & 300mg oral capsule	RM	2-way RM ANOVA	↓ (200 & 300mg)	
Fillmore et al., 2006a		Cue Dependent Go/No-go Task SST	N = 14 male & females with a history of cocaine use	Acute: 100, 200 & 300mg oral capsule	RM	2-way RM ANOVA	↓ Go/No-go (200 & 300mg) ↓ SST (100 & 200mg)	A U-shaped effect of cocaine on SSRT in the SST was demonstrated. In contrast a linear improvement in inhibitory control in the Go/No-go task was observed.
De Wit et al., 2000	D-AMPHETAMINE	SST	N = 20 Healthy male & females	Acute: 10 & 20mg oral capsule	RM	1-way RM ANOVA	↓ SST (10 & 20mg)	Inhibitory control increased, as demonstrated by the reduction in reaction time needed to inhibit an already initiated response. The reduction in impulsivity was restricted to with below average BL level of inhibitory control.

De Wit et al., 2002	SST Go/No-go Task	N = 36 Healthy male & female	Acute: 10 & 20mg oral capsules.	RM	1-way RM ANOVA	↓ SST (20mg) ↓ Go/No-go (10 & 20mg)	D-amphetamine decreased SRT in the SST without affecting Go reaction time suggesting a selective improvement in inhibitory control. D-amphetamine selectively reduced false alarms in the Go/No-go task without affecting reaction time. Positive effects on inhibitory control were only observed in participants with a low BL level of inhibitory control.
Fillmore et al., 2005	SST	N = 22 Healthy male & females	Acute: 7.5mg/70kg & 15mg/70kg	RM	2-way RM ANOVA	—	Participants displayed high levels of inhibitory control at BL, which may explain the lack of positive effect of the stimulant on performance.
Mulvihill et al., 1997	SST	N = 48 Healthy male & females	Acute: 620mg/kg (males) 540mg/kg (females)	RM	1-way RM ANOVA	↑	
Dougherty et al., 1999	CPT	N = 18 Healthy male & females	Acute: 3 hourly beverages of 200mg/kg	RM	1-way RM ANOVA	↑	
Fillmore & Vogel-Sprott, 1999	SST	N = 35 Health males	Acute: 620mg/kg beverage	BS	1-way ANOVA	↑	Selective reduction in inhibitory control, reaction time was unaffected under the influence of alcohol.

Finn et al., 1999	Go/No-go Task	N = 71 males & females with a positive family history of alcoholism N = 78 males & females with no family history of alcoholism	Acute: 0.07%, 0.09% breath alcohol level beverage	MD	2- way M ANOVA	↑ (0.07% & 0.09% in both individuals with and without a family history of alcoholism)	Findings also demonstrated that individuals with a low capacity working memory appear more sensitive to the effects of alcohol on inhibitory control.
De Wit et al., 2000	SST	N = 17 Healthy male & females	Acute: 200, 400 & 800mg/kg beverage	RM	1-way ANOVA	↑ (200 & 400mg/kg)	Decreased SSRT, without affecting frequency of commission errors.
Dougherty et al., 2000	CPT	N = 20 Healthy male & females	Acute: 500 & 100mg/kg beverage	RM	1-way ANOVA	↑ (500 & 100mg/kg)	Impairments in attention were additionally observed.
Easdon & Vogel- Sprott, 2000	SST requiring high or low information processing	N = 16 Healthy male social drinkers	Acute: 620mg/kg Beverage.	MD	2- way M ANOVA	↑ in both versions of SST	Selective effect, reaction times were unaffected by alcohol.

Marczinski & Fillmore, 2003a	Cue Dependent Go/No-go Task	N = 12 Healthy male & females	Acute: 450 & 650mg/kg beverage	RM	1-way RM ANOVA	↑ (450 & 650mg/kg only following presentation of incorrect go cue)	Alcohol only increased impulsive responding following the incorrect presentation of the Go cue during No-go trials.
Marczinski & Fillmore, 2003b	Cue Dependent Go/No-go Task	N = 12 Healthy male & females	Acute: 650mg/kg alcohol beverage; 2.0 & 4.0mg/kg caffeine; combination doses 2.0/ & 4.0/650 caffeine and alcohol	RM	3-way RM ANOVA	↑ (650mg/kg only following presentation of incorrect go cue)	Alcohol increased impulsive responding, an effect that was not antagonized by caffeine. Caffeine did however antagonize the effects of alcohol on slowing reaction time.
Ortner et al., 2003	Go/No-go Task	N = 76 Healthy males	Acute: 700mg beverage	BS	2-way ANOVA	—	
Fillmore & Weafer, 2004	Cue Dependent Go/No-go Task	N = 12 Health male & female social drinkers	Acute: 650mg/kg	RM	2-way RM ANOVA	↑ (only following presentation of incorrect go cue)	Greater deficits in inhibitory control were observed in males.
Easdon et al., 2005	Go/No-go Task	N = 24 Healthy male & females	Acute: 560 & 800mg/kg	BS	2-way ANOVA	↑ (550 & 800mg/kg)	

Marczinski & Fillmore, 2005	Cue Dependent Go/No-go Task	N = 17 Healthy male & female social drinkers	Acute: 450 & 650mg/kg	RM	2-way RM ANOVA	↑ (450 & 650 mg/kg only following presentation of incorrect go cue)	Slowed reaction times were additionally observed during performance of the task.
Marczinski et al., 2005	Cue Dependent Go/No-go Task. Where half of the participants executed a response during Go trials and the remaining participants disengaged from a response.	N = 24 Healthy male & female social drinkers	Acute: 450 & 650mg/kg	MD	2-way M ANOVA	↑ (450 & 650 mg/kg only following presentation of incorrect go cue)	The decrease in inhibitory control was only observed in the response engagement task and THE not response disengagement version of the task.
Reynolds et al., 2006b	SST Go/No-go Task	N= 24 Healthy male and female social drinkers	Acute: 400 & 800mg beverage	RM	2-way RM ANOVA	↑ SST (400 & 800mg) — Go/No-go Task	
Levin et al., 1996a	CPT	N = 11 Healthy non-smoker male & females	Acute: 7mg/day nicotine transdermal patch for 4.5 hours	RM	1-way RM ANOVA	—	Whilst nicotine did not affect levels of inhibitory control an improvement in attention was observed.

Levin et al., 1998	CPT	N = 15 Male & female smokers diagnosed with SCZ	Acute: 7, 14 & 21mg/day nicotine transdermal patch	RM	1-way RM ANOVA	↓ (21mg) ↑ (7mg)
Potter & Newhouse, 2004	SST	N = 8 Male & female adolescents diagnosed ADHD	Acute: 7mg/day nicotine transdermal patch for 60 minutes	RM	1-way RM ANOVA	↓
Bekker et al., 2005	CPT SST	N = 16 Healthy regular smokers following overnight abstinence	Acute: 7, 21mg/day nicotine transdermal patch for 60 minutes	RM	2-way RM ANOVA	— CPT — SST Whilst nicotine did not affect levels of inhibitory control an improvement in attention was observed in CPT
Mc Donald et al., 2003	THC SST Go/No-go Task	N= 37 Healthy male and female	Acute: 7.5 & 15mg oral capsule	RM	2-way RM ANOVA	↑ SST (15mg) — Go/No-go task

↑ Indicate that the drug increased disinhibition significantly in comparison to control treatment $p < 0.05$. ↓ Indicate that the drug decreased disinhibition significantly in comparison to control treatment $p < 0.05$. — Indicate that drug did not display any behavioural effects on disinhibition.

Abbreviations: ANOVA, Analysis of Variance; MD, Mixed Design; M, Mixed; MANOVA, Multivariate Analysis of Variance; BS, Between Subjects; RM, Repeated Measures; SST, Stop signal Task; SSRT Stop Signal Reaction Time; THC, Tetrahydrocannabinol

Table 1.4: The Acute and Chronic Effects of Drugs of Abuse on Inhibitory Control in Animal Subjects

<u>Authors</u>	<u>Drug</u>	<u>Paradigm</u>	<u>Sample</u>	<u>Dose</u>	<u>Design</u>	<u>Analysis</u>	<u>Effect</u>	<u>Comments</u>
Jentsh et al., 2002	COCAINE	Object Discrimination Task	N = 22 male & female Vervet monkeys	Chronic: treated with either saline, 2 or 4mg/kg i.m. for 14 days	MD	2-way M ANOVA	↑	Perseverative responding in the task, argued to be indicative of heightened disinhibition, increased following chronic treatment.
Paine et al., 2003		Asymmetrically Reinforced Go/no-go Task	N = 16 male LE rats	Chronic: treated with either saline or 15mg/kg i.p. three times per day for a 14 day period	MD	2-way M ANOVA	—	Reward contingencies were not balanced across Go and No-go trials. Disinhibition may increase in symmetrically reinforced Go/No-go Tasks.
Paine & Olmstead 2004		Asymmetrically Reinforced Go/no-go Task	N = 8 male LE rats	Acute: 5, 10, 15 & 20mg/kg i.p.	RM	2-way RM ANOVA	↑ (15mg/kg)	Increase in behavioral disinhibition appears to be independent of deficits in conditional discrimination or attention.
Cheng et al., 2006		DRL	N = 16 male SD rats	Acute: 15mg/kg i.p.	RM	1-way RM ANOVA	↑ (15mg/kg)	
Van Gaalen et al., 2006		5CSRTT	N = 12 male Wistar rats	Acute: 5, 10 & 20mg/kg i.p.	RM	1-way RM ANOVA	↑ (Across all doses)	
Ridley et al., 1980	D-AMPHETAMINE (METHAMPHETAMINE)	5CSRTT	N = 3 Marmosets	Acute: 0.4 & 0.8mg/kg i.p.	RM	1-way RM ANOVA	↑ (Across all doses)	

Harrison et al., 1997a	5CSRTT	N = 19 male LH rats	Acute: 0.2, 0.4 & 0.8mg/kg i.p.	RM	1-way RM ANOVA	↑ (0.4 & 0.8mg/kg)	
Evenden 1998a	FCN 8	N = 16 male SD rats	Acute: 0.4, 0.8, 1.6 & 2.4mg/kg s.c.	RM	---	↑ (0.8-2.4mg/kg)	
Evenden 1998b	FCN8	N = 8 male SD rats	Acute: 0.2, 0.4, 0.8, mg/kg s.c.	RM	1-way RM ANOVA	↑ (0.8mg/kg)	
Foela et al., 2000	SST	N = 11 male SD rats	Acute: 0.125, 0.25, 0.5 & 1.0mg/kg s.c.	RM	1-way RM ANOVA	↓ (0.25 & 0.5mg/kg)	Improved inhibitory control only in those animals exhibiting a low BL level of task and at performance. Only observed at low to moderate doses.
Wiley et al., 2000	DRL	N = 12 male LE rats	Acute: 0.1, 0.3 1.0 & 3.0mg/kg s.c.	RM	1-way RM ANOVA	↑ (1.0mg/kg)	Increase in burst responses may be mediated by amphetamines known effect on time perception.
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Evenden 1998a	FCN 8	N = 16 male SD rats	Acute: 100, 300, 1000 & 3000mg/kg i.p..	RM	1-way RM ANOVA	↑ (1000 & 3000mg/k g)	The effect however was not selective, a decrease in response rate in the task was observed.
Evenden 1998b	FCN 8	N = 8 male SD rats	Acute: 300, 1000 & 3000mg/kg i.p..	RM	1-way RM ANOVA	↑ (3000mg/k g)	The effect however was not selective, a decrease in response rate in the task was observed.

Foela et al., 2000	SST	N = 11 male SD rats	Acute: 250, 500 & 700mg/kg s.c.	RM	1-way RM ANOVA	↑ (500 & 700mg/kg)	A selective effect was observed. Ethanol impaired inhibition without affecting reaction time on the task.
Morrison et al., 1968	DRL	N = 8 male LH rats	Acute: 0.06, 0.12, 0.25, 0.5, 1.0 & 2.0mg/kg	RM	1-way RM ANOVA	↑ (0.12, 0.25, 0.5 & 1.0mg/kg)	
Bizot, 1998	DRL	N = 24 male W rats	Acute: 0.05, 0.1, 0.2 & 0.4 mg/kg s.c.	RM	1-way RM ANOVA	↑ (0.2, 0.4mg/kg)	
Mirza & Stolerman, 1998	5CSRTT	N = 28 male LH rats	Acute: 0.05, 0.15, & 0.4 mg/kg s.c.	RM	-----	↑ (0.05, 0.15 & 0.4mg/kg)	Dose dependent increase in impulsive responding under low event rate conditions.
Blondel et al., 1999	5CSRTT	Acute N = 12 Chronic N = 22 male SD rats	Acute: 0.03, 0.1 & 0.3mg/kg i.p. Chronic: 0.1, 0.3mg/kg i.p. for 5 consecutive days	Acute: RM Chronic: MD	1-way RM ANOVA	— Acute ↑ Chronic	Response latencies during chronic drug treatment decreased significantly. The increase in impulsive responding may be attributed to nicotine induced sensitization of motor function.

Blondel et al., 2000	5CSRTT	N = 22 male SD rats	Chronic: 0.3mg/kg i.p. administered 3 times over 7 days. A fourth 0.3mg/kg dose was administered 2 weeks later.	MD	Acute: 1-way RM ANOVA Chronic: 2-way M ANOVA	↑ (Third dose, an effect still observed 2 weeks later)	Although initial exposure to nicotine was without effect on impulsive responding, repeated treatment led to an increase in disinhibition. RTs, however, were also reduced. It is therefore unclear whether the observed effect could instead reflect nicotine induced hyper motor function.
Grottick & Higgins, 2000	5CSRTT	N = 5 male LH rats	Acute: 0.2mg/kg s.c.	RM	2-way RM ANOVA	↑	
Popke et al., 2000a	DRL	N = 21 male SD rats	Acute: 0.3, 0.42, 0.56, 0.75 & 1.0mg/kg i.p.	RM	1-way RM ANOVA	↑ (0.3 & 0.56 mg/kg)	
Stolerman et al., 2000	5CSRTT	N = 8 male LH rats	Acute: 0.05, 0.1 & 0.2mg/kg s.c.	RM	1-way RM ANOVA	↑ (Across all doses)	Impulsive responding increased across the dose range tested under conditions of high attentional demand. Both ITI and stimulus duration was varied during test session.

Grottick & Higgins, 2001	5CSRTT	N = 26 male SD rats	Chronic: saline or 0.2mg/kg administered once per day for 20 days followed by acute assessment of nicotine at 0.1, 0.2 & 0.4mg/kg s.c.	RM	2-way RM ANOVA	↑ (0.2mg/kg)	An increase in locomotor activity was additionally observed. It is difficult to dissociate nicotine's effects on sensitization on motor function from that of inhibitory control.
Hahn et al., 2002	5CSRTT	N= 32 male LH rats	Acute: 0.05, 0.1, 0.2 & 0.4mg/kg s.c.	RM	2-way RM ANOVA	↑ (0.05, 0.1 & 0.2mg/kg)	
Bizarro et al., 2004	5CSRTT	N = 24 male LH rats	Acute: 0.025, 0.05, 0.1 & 0.2 mg/kg s.c.	RM	1-way RM ANOVA	↑ (across all doses)	Prior to the assessment of the dose response of nicotine animals were treated on three with nicotine occasions to reduce initial depressed motor effects.
Bruin et al., 2006	5CSRTT	N = 41 male C57BL/6J mice	Acute: 0.08, 0.16 & 0.31mg/kg s.c.	BS	MANOVA	↑ (0.08mg/kg)	
Day et al., 2007	5CSRTT	N = 15 male LH rats	Acute: 0.05, 0.1, 0.2 & 0.4mg/kg s.c.	RM	1-way RM ANOVA	↑ (across all doses)	Impulsive responding was only increased in animals previously exposed to nicotine. No effects of nicotine were observed in drug naïve animals.

Wiley et al., 2000	THC	DRL	N = 12 male LE rats	Acute: 0.3, 1.0, 3.0, 10.0 & 30.0mg/kg s.c.	RM	1-way RM ANOVA	↑ (1.0 & 3.0mg/kg)
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↑ Indicate that the drug increased disinhibition significantly in comparison to control treatment p< 0.05. ↓ Indicate that the drug decreased disinhibition significantly in comparison to control treatment p< 0.05. — Indicate that drug did not display any behavioural effects on disinhibition. . . — No information available

Abbreviations: ANOVA , Analysis of Variance; BS, Between Subjects; i.p, intraperitoneal; LE, Long Evans; LH, Lister Hooded; MD, Mixed Design; M, Mixed; MANOVA Multivariate Analysis of Variance; RM, Repeated Measures; s.c., subcutaneous; SD, Sprague Dawley ; W, Wistar

1.3.2.6. Examination of the Effects of Acute Withdrawal on Impulsivity

Only a limited number of studies have explored the effect of acute drug withdrawal or drug deprivation on impulsive choice and to an even lesser extent, inhibitory control. For example Giordano and colleagues (2002) compared in opioid dependent patients, maintained on buprenorphine, rates of discounting of delayed reward when both mildly opioid deprived and opioid sated. When opioid deprived, subjects discounted both delayed heroin and monetary rewards to a greater extent than when opioid sated. Heroin was discounted to the greatest degree during both stages, with discounting rates shown to be two to three times more rapid than that of delayed monetary rewards.

Comparable findings have since been observed in short term deprived smokers (Field et al., 2006). Smokers deprived for a period of 13h discounted both delayed hypothetical monetary and cigarettes to a greater degree relative to when completing the task 5 minutes following the smoking of a cigarette. Once again, the drug of abuse displayed the steepest rates of discounting. Inconsistent with these findings, smokers deprived of nicotine for 24h demonstrated no evidence of an increase in intolerance to delayed reward when choices were made regarding monetary rewards (Mitchell 2004). Impulsive choice in Mitchell's study was only increased for cigarettes during acute deprivation, leading authors to conclude that findings demonstrated more of an increased preference for cigarettes rather than evidence of a greater intolerance to delay. The discrepancy in findings in smokers can possibly be attributed to both the lack of power (N= 11) in Mitchell's study, and the variation in a DD tasks utilised across studies.

In models of inhibitory control, both overnight and 24h smoking abstinence was associated with a poorer ability to withhold inappropriate responding on the CPT (Hatsukami et al., 1989; Dawkins et al., 2007). This impaired performance furthermore was attenuated by administration of nicotine (Dawkins et al., 2007).

In the animal literature reports have only been made on the effects of drug withdrawal on inhibitory control. In rats, Dalley and co-workers (2005a) demonstrated no significant changes in impulsive responding in the 5CSRTT when tested at 24 hours post termination of six consecutive days of cocaine self administration. Comparably, in the same task a lack of effect on impulsivity has also been shown following the withdrawal from both heroin and amphetamine intravenous self administration (Dalley et al., 2005a; 2005b). More recently, a further study examined differences in response to intermittent withdrawal from cocaine between high and low trait impulsive animals based on the level of anticipatory responding in the 5CSRTT. Findings demonstrated a contrasting normalisation in impulsive responding in high trait impulsive animals relative to the low impulsive group (Dalley et al., 2007). Within the

animal literature it has yet to be elucidated whether comparable effects of drug withdrawal will be observed in animal models that are designed more specifically to measure behavioural inhibition, or models that assess different forms of impulsive behaviour such as sensitivity to delayed reward.

The augmented levels of impulsivity, in particular impulsive choice, during early withdrawal may play an important role in the maintenance and relapse of drug dependence. The deficits in self control during early abstinence may lead to the increased likelihood of dependent individuals choosing once again the immediate rewarding effects of the drug, or relief of withdrawal as the larger delayed rewards associated with a drug free lifestyle will be discounted to the greatest extent during this stage. As a result, attempts to abstain will continue to fail and drug taking behaviour will be maintained. Interestingly when alcohol and cocaine dependent individuals were asked the subjective reasons for relapse, 41% reported it was an 'impulsive action', or loss of control, with only 7% reporting reasons of craving (Miller and Gold, 1994). It is crucial that future research explores further the role of impulsivity during acute withdrawal which may aid further our understanding of cessation failures amongst dependent individuals. Indeed recent research is beginning to highlight the importance of impulsivity in relapse and thus demonstrating the essentiality of the need of both behavioural and pharmacological interventions to target impulsivity in the treatment of substance dependence. For example, alcoholics who displayed greater deficits in inhibitory control were more likely to relapse during the two months following a detox programme (Noel et al., 2002). Comparably, the level of impulsive choice and disinhibition, as assessed on the EDT and CPT respectively, was found to be significantly greater in adolescents who failed to successfully abstain from smoking during a fourteen week smoking cessation programme (Krishnan-Sarin et al., 2007).

Findings of the effect of short term deprivation on impulsive responding if taken together with results from research assessing longer term abstinence suggest that perhaps a biphasic relationship between abstinence and impulsivity may exist, at least in the case with impulsive choice behaviour (Reynolds, 2006b). Initial abstinence from the drug and early onset withdrawal may result in a heightening of impulsivity, during which time individuals are at a potentially higher risk of relapse. With continued abstinence levels of impulsive responding may then begin to decrease (e.g. Bickel et al., 1999; Petry et al., 2001). Such conclusions cannot however be made until research assesses impulsivity over time; commencing at the point of initial withdrawal and continuing through to long term abstinence within subjects.

1.3.2.7. Examination of Impulsivity as a Predictor of Drug Abuse

Research that has explored the theory that high levels of impulsivity may predispose the abuse of drugs is limited. In a study that explored the predictors of cigarette smoking in adolescence,

sensitivity to delayed reward at 9th grade was not associated with the progression of smoking during the following four years (Audrian-McGovern et al., 2004). DD however did indirectly influence the initiation of smoking through complementary reinforcers such as peer smoking, alcohol and drug use, which alone increased the likelihood of smoking. This may suggest that sensitivity to delayed gratification may be more of a risk factor for the use of other drugs of abuse. Whilst DD was not a predictor of future smoking, at baseline sensitivity to delayed reward was significantly higher in adolescents who were currently regular smokers, supporting the theory that perhaps impulsivity is instead augmented by smoking (Audrian-McGovern et al., 2004).

In a further study that assessed the role of disinhibition, findings demonstrated that whilst the level of inhibitory control at the age 10-12 years did not influence the use of drugs at 16, it did significantly predict the development of substance use disorder at 19 years of age (Tarter et al., 2003). These findings suggest that this subcomponent of impulsivity may play little role in the initiation of substance taking, but instead significantly predict the future progression to substance use disorder once these substances have been used. In a comparable study Aytyacler et al., (1999) provided evidence that impaired executive functioning in childhood, which included the impaired ability to inhibit responding, was a valid predictor of substance misuse in adolescence. More specifically, smoking, cannabis use, and the number of drugs that had been experimented were reliably predicted.

Evidence has additionally been provided in the animal literature that impulsivity may predispose the abuse of drugs. Baseline levels of sensitivity to delayed reward and levels of anticipatory responding, as measured in a adjusting amount DD procedure and 5CSRTT respectively, have both predicted levels of cocaine self-administration in rats (Perry et al., 2005; Dalley et al., 2007). Higher trait levels of impulsivity on both measures led to greater rates of cocaine self administration relative to the low impulsive group. Comparably, animals exhibiting the greatest intolerance to delayed reward consumed significantly more freely available ethanol compared to both medium and low level impulsive groups (Poulos, Le and Parker, 1995). Furthermore, mice strains that exhibited more impulsive responding on an appetitive signalled nose poke task, a paradigm comparable to the 5CSRTT, also drank more ethanol (Bowers and Wehner, 2001).

Taken together, these findings suggest that heightened impulsivity may precede and act as predisposing factor to substance misuse and possibly substance use disorder. The difficulties associated with administering behavioural tasks in longitudinal research combined with the lack of knowledge concerning the stability of performance in such tasks has meant that research in this area is lacking considerably (Mitchell, 2004). The strongest argument that impulsivity may be a risk factor of substance dependence comes instead from indirect evidence that indicates

children diagnosed with impulse control problems, such as ADHD and conduct disorder, are significantly more likely to abuse both licit and illicit agents (e.g. Biederman et al., 1997; Burke, Loeber and Lahey, 2001; Moss and Lynch, 2001; Molina and Pelham, 2003). If one therefore considers the evidence together, it is highly probable that heightened impulsivity may act as predisposing factor drug dependence and therefore highlights the importance of future prevention strategies to consider managing both impulsive choices and disinhibited behaviour.

1.4. NEUROBIOLOGICAL MECHANISMS OF IMPULSIVITY AND ITS RELATION TO DRUG DEPENDENCE

An abundance of research has focused upon revealing the neural mechanisms underlying impulsivity. In the following sections both the neurochemical and neuroanatomical evidence will be reviewed and how these mechanisms may modulate the heightened impulsivity associated with drug dependence additionally explored.

1.4.1. Neurochemical Basis of Impulsivity

1.4.1.1. The Role of Serotonin

One of the most predominant neurobiological theories of impulsivity argues that serotonin (5-HT) plays a central role in its mediation, whereby an inverse relationship exists between 5-HT functioning and impulsive behaviour (e.g. Soubri , 1986; Logue, 1988). Drive for this theory came from the influential studies of Linnoila (1983) on violent behaviour and Asberg et al., (1976) on suicide, who argued that low levels of serotonergic function may be related to pathological impulsivity. Linnoila (1983) found that cerebrospinal fluid (CSF) 5-hydroxytryptamine (5-HIAA) was reduced in aggressive individuals only if the aggression was impulsive. Similar findings were reported in suicide attempters with depression (Asberg et al., 1976), violent suicide attempters (Coccaro, 1989) children with conduct disorders (e.g. Stoff et al., 1987) and normal individuals with high impulsivity as a personality trait (Roy et al., 1988). Further support is provided by findings in non-human primates where aggression and impaired impulsive control is associated with lower levels of 5-HT (Raleigh, McGuire, Brammer and Yuwiler, 1984; McGuire and Raleigh, 1987; Higley et al., 1997; Westergaard, Suomi, Higley and Mehlman, 1999).

Research that has directly manipulated 5-HT activity has additionally provided, to some extent, evidence of the neurochemical substrate's implication in impulsive behaviour. In animal models of delayed reward, dysfunction of the 5-HT pathways has been associated with alterations in impulsive behaviour in rats (Ho et al., 2002). Bizot et al. (1999) demonstrated that forebrain 5-HT depletion resulted in an increase in the choice of the smaller immediate reward in comparison to sham lesioned rats. Moreover, in the T-maze, the lesion was found not to affect the discrimination between the magnitude of the two rewards. These findings are

consistent to those of Thiebot (1992) where a reduction of 5-HT, induced by p-chlorophenylalanine (PCPA), increased impulsive behaviour in the same model. In operant paradigms of delayed reward parallel findings have also been shown. Reduced concentrations of 5-HT and its metabolite 5-HIAA, induced by 5,7-dihydroxytryptamine (5,7-DHT), increased preference for the smaller immediate reward in comparison to the sham-lesioned controls (Wogar, Bradshaw and Szabadi, 1993; Richards and Seiden, 1995; Ho et al., 1998; Al-Ruwaitea et al., 1999; Mobini et al. 2000a; 2000b). Findings furthermore, suggest that the increase in impulsive choice demonstrated can be attributed to the increases in sensitivity to delay rather than changes in sensitivity to reward magnitude (Ho et al., 1999; Mobini et al., 2000b). Conversely, enhancing 5-HT function, through either augmenting its release (d-fenfluramine) or by selectively blocking its reuptake (fluvoxamine, fluoxetine, clomipramine, zimeldine) has been shown to increase preference for the delayed larger reward in the T-maze procedure in rats (Bizot et al., 1988; Poulos, Parker and Le, 1996; Bizot et al., 1999). More recently, further evidence of a decrease in impulsivity has been shown following 17 days of chronic treatment with the selective serotonin reuptake inhibitors (SSRIS) fluoxetine, citalopram and paroxetine in an adjusted delay paradigm in pigeons (Wolff and Leander, 2002). Comparably, Adriani et al. (2004) demonstrated that increasing 5HIAA/5-HT ratio in medial frontal cortex and cingulate cortex through chronic treatment of acetyl-L-carnitine led to a decrease in impulsive choice in animals highly sensitive to delayed gratification at baseline.

Findings however have not been entirely consistent. The heightened impulsivity demonstrated following infusion of 5,7-DHT has in some instances only been a temporary effect (e.g. Bizot et al., 1999) and in more recent studies global 5-HT depletion failed to alter impulsive choice (Winstanley et al., 2003; 2004b; 2005). Charrier and Thiebot (1996) also failed to demonstrate an effect of PCPA on choice behaviour in a “systematic” operant delayed reward task. In direct opposition, Evenden and Ryan, (1996) actually displayed a decrease in impulsive choice following treatment with the non-selective 5-HT antagonist metergoline. A number of differences across studies may account for the discrepancy in finding, including varying DD paradigms and methodological procedures. For instance, research that demonstrated a lack of effect, explored the effects of 5-HT depletion on performance following the acquisition of a “systematic” DD task (Winstanley et al., 2003; 2004b). In contrast, research that reported significant increases in discounting of delayed reward adopted “adjusting” DD paradigms and examined the effect of depletion during the acquisition of the paradigm (e.g. Wogar et al., 1993; Mobini et al., 2000a; 2000b).

Manipulation of 5-HT activity has additionally provided support for the role of 5-HT in behavioural disinhibition. Global decreases in serotonergic function in rodents have been shown to increase impulsive responding during No-go trials in both asymmetric (Fletcher, 1993) and

symmetric go/no-go paradigms (Harrison, Everitt and Robbins, 1999). Further comparable findings of induced disinhibition following 5-HT depletion have been observed in DRL (e.g. Thornton and Goudie, 1978; Wogar, Bradshaw and Szabadi, 1992; 1993), FCN procedures (Evenden, 1998a) and the 5CSRTT (Harrison et al., 1997a; 1997b; Winstanley et al., 2004d). Whilst serotonergic disruption in timing behaviour may account for increased premature responses in DRL procedure (Wogar et al., 1992; Morrissey et al., 1993), this interpretation cannot explain the impaired control over responding observed during the go/no-go task. In the latter of these tasks, explicit visual exteroceptive cues are available indicating when to respond or withhold a response, thus eliminating the need for timing and allowing clearer conclusions to be reached on the role of 5-HT on response disinhibition (Harrison et al., 1999).

Further support for the role of 5-HT in the modulation of response inhibition is evident from the decrease in premature responding in rodents following treatments that enhance 5-HT neurotransmission. The SSRI, fluoxetine, decreased early responding in a DRL task (Richards et al., 1993) whilst monoamine reuptake inhibitor, imipramine, reduced impulsive responding in the FCN procedure (Evenden, 1998b; but see also Evenden, 1998a). The latter finding implies the possibility that stimulation of noradrenergic and dopaminergic in addition to serotonergic systems may be implicated in this aspect of impulsivity.

In human studies the effects of 5-HT reduction have additionally been examined by methodology of dietary rapid tryptophan depletion (RTD) which effectively decreases 5HT metabolites in CSF (e.g. Williams et al., 1999). A reduction in 5-HT has decreased inhibitory control on a range of tasks including the SST, CPT and go/no-go task in healthy males and in subjects with a family history of alcoholism (Le Marquand et al., 1999; Crean, Richards and de Wit, 2002; Walderhaug et al., 2002; Walderhaug et al., 2005; but see also Clark et al., 2005). In terms of the effects of RTD on impulsive decision making, no significant effects have been reported in a hypothetical DD paradigm (Crean et al., 2002). Taken together, following 5-HT depletion more consistent increases in disinhibition have been demonstrated relative to the effects observed on impulsive decision making. On this basis, it could be argued that findings support the theory that these two components of impulsivity are perhaps neurobiologically dissociated (Evenden, 1999), whereby the serotonergic system plays a more prominent role in the modulation of inhibitory control. However, the animal research that has implicated 5-HT in impulsive choice cannot be ignored. Although no effect of serotonergic manipulation was observed in human participants, this may be accounted for by the hypothetical version of the task being less sensitive to acute state changes in impulsivity induced by RTD. As previously highlighted human tasks such as the EDT where rewards and their delay to their delivery are experienced, display greater sensitivity to acute drug effects than hypothetical DD paradigms

(Reynolds et al., 2006b). 5-HT manipulations may therefore induce more substantial effects on impulsive choice in such paradigms and should be an essential avenue of future research.

Research that has, however, examined selective serotonergic drugs have demonstrated alterations in both sensitivity to delayed gratification and behavioural inhibition. These effects are complex and suggest that 5-HT may modulate varying aspects of impulsive behaviour through mediation of different receptor subtypes. However, activation of the 5-HT_{1A} receptor has yielded comparable results on both components of impulsivity. In the DD paradigm in rodents, increased choice of the immediate reward has been reported following the administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2(di-n-propylamino) (8-OH-DPAT), in both a “systematic” DD paradigm (Winstanley et al., 2005) and the T-maze procedure (Poulos et al., 1996). Evenden and Ryan (1999) furthermore demonstrated that 8-OH-DPAT can increase preference for the immediate reward; the effect however was restricted to the beginning of the test session, during which the delayed larger reward was delivered following shorter time periods. At higher delays a contrasting decrease in impulsive choice was shown (Evenden and Ryan, 1996). A comparable delay related effect has since been replicated with the 5-HT_{1A} receptor agonist flesinoxan (van den Bergh et al., 2006).

Partial agonists at this receptor such buspirone, ipsapirone and MDL 73055EF, have additionally displayed evidence of induced heightened impulsive decision making following acute administration (Bizot et al., 1999; Liu et al., 2004), whilst following chronic buspirone an opposite decrease in impulsivity has been shown (Liu et al., 2004). The evident increase in impulsive choice following the acute administration of 5-HT_{1A} full and partial agonists can possibly be attributed to the decrease in 5-HT neurotransmission via the agonist activation of the somatodendritic 5-HT_{1A} autoreceptors (e.g. Sprouse and Aghajanian, 1988; Bonvento et al., 1992). The contrasting decrease in impulsive choice following the chronic administration of buspirone is difficult to interpret, but may be attributed to the sensitisation of 5-HT_{1A} post synaptic receptors following chronic exposure (De Vry, 1995). To date it is unknown whether comparable anti-impulsive choice effects are shown following the chronic treatment of other 5-HT_{1A} agonists.

Treatment doses of 8-OH-DPAT in the tasks that assess behavioural disinhibition have additionally provided evidence of heightened impulsive choice. Injection into the median raphe nucleus disinhibited responding in a go/no-go operant procedure (Fletcher, 1993) whilst subcutaneous administration of the agonist increased premature responding in 5CSRTT via action at the presynaptic 5-HT_{1A} receptors (Carli and Samanin, 2000). Mixed results however have been yielded following the administration of the 8-OH-DPAT in FCN procedures in rats, with both an increase (Mele et al., 1994) and decrease (Evenden, 1998c) in impulsive

responding been obtained. Furthermore, Evenden (1998c) displayed an increase in premature responding following antagonism of the 5-HT_{1A} receptor with WAY100635. The discrepancy in findings was attributed to the contrasting paced (Evenden, 1998c) and unpaced (Mele et al., 1994) FCN procedures adopted across studies.

Increasing interest has also been given to the potential role of the 5HT_{1B} receptor in the mediation of impulsivity, with knockout mice lacking this receptor subtype demonstrating heightened levels of impulsive behaviour (Brunner and Hen, 1997). Since then, activation of this receptor by the agonist eltoprazine, has demonstrated an increase preference for the larger delayed reward, however the effect was modest and found only at one dose tested (van den Bergh et al., 2006). In contrast, following stimulation of this receptor by the 5HT_{1A/B} agonist RU-24969 in a model of response inhibition, the FCN, an increase in premature responding was observed (Evenden, 1998c). The opposing effects in each of these models of impulsivity corroborate perhaps the neurochemical dissociation of differing aspects of impulsivity at this receptor (Evenden, 1999).

Subtypes of the 5-HT₂ receptor have additionally been studied in animal models of impulsivity. The 5-HT₂ agonist DOI has been reported to increase impulsive responding across paradigms. Enhanced sensitivity to delayed reward (Evenden and Ryan, 1999), increased premature responding in 5CSRTT (Koskinen et al., 2000a; Koskinen and Sirvio, 2001), and reduction in length of responses in an FCN procedure (Evenden, 1998c) have been observed following its administration. Furthermore, blockade of this receptor subtype, by the 5-HT_{2A/C} antagonist ketanserin, has displayed evidence of a decrease in premature responding in the 5CSRTT following its administration both peripherally or into the medial frontal cortex (Koskinen et al., 2000a; 2000b; Passetti et al., 2003). In contrast, no significant modification in performance was earlier reported following the administration 5-HT₂ antagonist, ritanserin, on a delayed reward task (Evenden and Ryan, 1999). A more recent study by Talpos and co-workers (2006) however, demonstrated differential effects of 5-HT₂ antagonists on the two distinct measures of impulsivity. As previously demonstrated, the administration of 5-HT_{2A/C} antagonist ketanserin led to a decrease in premature responding in the 5CSRTT, an effect however that was not observed in a delayed reward paradigm. Conversely, the 5-HT_{2C/B} antagonist, SER-082, decreased impulsive decision making without significantly affecting premature responding in the 5-CSRTT. Research has shown that ketanserin and SER-082 can be administered to dissociate the role of the 5-HT_{2C} subtype receptor from 5-HT_{2A} on measures sensitive to serotonergic manipulation (e.g. Currie et al., 2002). On this basis authors concluded that evidence had been provided that independent components of impulsivity are mediated by differing neurochemical substrates, supporting further their dissociation at a behavioural level. Findings suggest that the 5-HT_{2A} receptor is implicated in behavioural inhibition, a theory

further supported further by the decrease in premature responding in 5CSRTT shown following selective blockade of this receptor by M100907 (Higgins et al., 2003; Winstanley et al., 2003b; 2004d). Sensitivity to delayed reward instead appears to be modulated by the 5-HT_{2C} receptor subtype (Talpos et al., 2006). It is important to also to highlight that investigations of the 5-HT₂ receptor subtypes have provided findings that are contrary to the general theory that heightened impulsivity is associated with decreased serotonergic function (e.g. Soubri , 1986; Harrison et al., 1997; Mobini et al., 2000a). Clearly the role of 5-HT in the mediation of impulsivity is complex. The fact that these receptors are also involved in the regulation of DA activity (e.g. Di Matteo et al., 1998; 1999; Gobert and Millan, 1999), may begin to explain the discrepancy in findings and suggest more importantly that a complex interaction between DA and 5-HT may exist in the modulation of impulsive behaviour (e.g. Winstanley 2003b).

Exploration of the involvement of further 5-HT receptor subtypes has indicated minimal or no evidence of their potential role in the modulation of impulsivity. Antagonism of the 5-HT₃ receptor had no effect on either impulsive choice or response inhibition as measured by a discrete trial delayed reward task and FCN procedure (Evenden 1998c; Evenden and Ryan, 1999). The 5-HT₆ receptor antagonist, SB-270146-A, furthermore displayed no modification on premature responding in the 5CSRTT or sensitivity to delayed reward following its systemic administration (Talpos et al., 2006).

Upon reviewing the research, there is strong evidence suggesting that impulsivity is related to impaired 5-HT functioning. The underlying mechanisms however are considerably more complex than previously acknowledged (e.g. Soubri , 1986), with perhaps a dissociation of the role of 5-HT in differing aspects of impulsivity at the receptor subtype level. With alterations in serotonergic function being recognised as a core mechanism of drug addiction (e.g. Koob, 2000), it may therefore be via this neurochemical mechanism that dysfunctions impulsive control are mediated in this population. Drugs of abuse including cocaine (e.g. Andrews and Lucki, 2001; Mangiavacchi et al., 2001), amphetamine and its derivatives (e.g. Kuczenski et al., 1995; Millan et al., 1999; Hedou et al., 2000), MDMA (e.g. Kankaanpaa et al., 1998), nicotine (e.g. Ribeiro et al., 1993; Summer et al., 1996) and alcohol (e.g. Weiss et al., 1996; Uzbay, Usanmaz and Akarsu, 2000) have demonstrated increases in extracellular levels of 5-HT in several brain regions following their administration. In contrast, at baseline drug dependent individuals display reduced levels of 5-HT functioning. Lower baseline 5-HT has been observed in excessive alcohol drinkers (Fils-Aime et al., 1996), whilst evidence of reduced peripheral 5-HT activity has been shown in cocaine abusers (Patkar et al., 2003a) and heavy smokers (Patkar et al., 2003b). Similarly, an earlier study by von Knorring and colleagues (1984) demonstrated that low platelet monoamine oxidase (MAO) activity, a biological marker of 5-HT activity, was associated with greater tobacco and alcohol use and as well as symptoms of possible alcohol

dependence. With evidence implicating also lowered 5-HT functioning in impulsivity, it is likely therefore that the 5-HT dysfunction evident in abusers may play a mediating role in the associated augmented impulsivity. In support of this theory, self reported BIS scores of impulsivity in both cocaine and heavier smokers, were found to be negatively correlated with 5-HT transporter (5-HTT) availability (Patkar et al., 2003a; 2003b). It remains unclear from such research however, whether the 5-HT abnormalities precede drug abuse, or are instead a consequence of chronic drug exposure. If the former of these theories is true, then 5-HT dysfunction may therefore represent a possible biological trait marker for heightened impulsivity and risk for the development of addiction. If instead drug use leads to 5-HT abnormalities, then this may mediate the subsequent loss of inhibitory control and impulsive choice in abusers, leading to maintenance and relapse of the disorder. Support for the latter direction has been provided from evidence indicating that chronic treatment with addictive drugs, such as cocaine, has been associated with a decrease in neuronal response to 5-HT neurotransmission (e.g. Levy et al., 1993; Buydens-Branchey et al., 1999).

Irrespective of the direction of the relationship, evidence of a reduction in impulsivity following the enhancement of 5-HT neurotransmission provides a potential pharmacological treatment mechanism for reducing impulsivity in substance abuse (e.g. Wolff and Leander, 2002). Indeed, preclinical research has demonstrated that increasing levels of 5-HT attenuates self administration of drugs such as amphetamine (eg. Lyness, 1983), cocaine (eg. Carroll et al., 1990; Glatz et al., 2002) alcohol (e.g. Zabik et al., 1982; Naranjo and Knoke, 2001) and nicotine (Opitz and Weischer, 1988). The reduction in self administration of these drugs may possibly be via a strengthening of self control. To date however, no strong evidence has yet been provided to demonstrate the efficacy of SSRIs in treatment of substance abuse disorder, despite evidence of their ability to reduce both impulsive choice and inhibitory control (e.g. Grabowski et al., 1995; Kranzler et al., 1995; Ciraulo et al., 2005). The serotonergic neuroadaptations associated with long term drug exposure could potentially reduce the effectiveness of such medications on reducing impulsivity in long term drug abusers.

Each of the 5-HT receptor subtypes argued to play a role in the mediation of impulsivity have furthermore been implicated in the neurobiological mechanisms of drug addiction, in particular the 5-HT_{1A} receptor (e.g. Rocha et al., 1998; Filip, Nowak, Papla, 2001; Davidson et al., 2002; Muller and Cary, 2006; Muller et al., 2007). For example, stimulation of the 5-HT_{1A} receptor by the agonist 8-OH-DPAT has been shown to facilitate cocaine administration and ethanol drinking whilst antagonism of this receptor has demonstrated an attenuation of both these behaviours (e.g. Schenk, 2000; McKenzie-Quirk and Miczek, 2003; Czoty et al., 2005). The enhancement of impulsive responding following activation of this receptor suggests that perhaps the observed augmentation of drug self administration may in part be via an induced loss of

control over behaviour. Blockade of this receptor may therefore offer a potential therapeutic target that would decrease abuse through increasing self control.

1.4.1.1. The Role of Dopamine

Research examining the pharmacological basis of impulsivity has additionally assessed the role of DA. The effect of increases in synaptic levels of DA has been most widely studied through the administration of the powerful dopaminergic agonist, amphetamine. As previously discussed, in both the human and animal literature acute doses of d-amphetamine have yielded mixed results in delayed reward models; both increases (Charrier and Thiebot, 1996; Evenden and Ryan, 1996; Cardinal et al., 2000; Isles et al., 2003; Helms et al., 2006) and decreases (Richards et al., 1999a; 1997; Cardinal et al., 2000; Wade et al., 2000; de Wit et al., 2002; Winstanley et al., 2003a; van Gaalen et al., 2006) in impulsivity have been found. Chronic administration of methamphetamine, which is more comparable to the pattern of drug use in dependent individuals, has however been found to promote choice of the immediate smaller reward in rodents (Richards et al., 1999a). The contradictory findings following acute treatment were attributed to a number of procedural differences across studies, including differences in rewards utilised, dose level (e.g. Isles et al., 2003) and presence or absence of a reward-predicting cue during the delay to delivery of the larger reward (Cardinal et al., 2000).

Evidence of a relationship between behavioural disinhibition and DA has in addition been provided. In human subjects increasing synaptic levels of DA, through administration of low to moderate doses of d-amphetamine, led to a significant increase in inhibitory control in the go/no-go task and SST procedures. This effect, however, was restricted to individuals with a poor baseline level of inhibitory control (de Wit et al., 2000; 2002). Comparable findings have been demonstrated in the animal literature following low doses of 0.25mg/kg and 0.5mg/kg of d-amphetamine in a rat version of the SST (Feola et al., 2000). In contrast, strong evidence has been provided in rodents that higher doses of the DA agonist, as in the DD paradigm (e.g. Isles et al., 2003), has an opposite effect on inhibitory control. Significant increases in impulsive responding in the DRL (Wiley, Compton and Golden, 2000), FCN (Evenden 1998a; 1998b), asymmetrical go/no-go (Ridley et al., 1980) and 5CSRTT procedures (Cole and Robbins, 1987; Harrison et al., 1997; Van Gaalen et al., 2006) have been demonstrated.

The ability of amphetamine to alter levels of both forms of impulsivity has however been shown to be partially dependent on serotonergic neurotransmission. The ability of the psychostimulant to decrease impulsive choice in a delayed reward paradigm was found to be significantly attenuated in ICV 5,7-DHT lesioned animals (Winstanley et al., 2003a). In a further study, although an opposite increase in premature responding in 5CSRTT was shown following amphetamines administration, 5-HT neurotransmission was once again essential for the

expression of amphetamines effects (Harrison et al., 1997). These data suggest that both sensitivity to delayed reward and behavioural inhibition are modulated through complex interactions between the 5-HT and DA systems.

Few studies have investigated the neurochemical basis of impulsive choice at the level of individual DA receptors. The systemic administration of the D2 antagonist raclopride and the D1/D2 antagonist flupenthixol (Cardinal et al., 2000; Wade et al., 2000), but not D1 antagonist SCH23390 (Wade et al., 2000), increased preference for the immediate, smaller reward, supporting the importance of the possible role of the D2 receptor in the intolerance to delayed reward. More recently however, in a systematic DD paradigm, impulsivity was increased following the administration of D1 antagonist SCH23390 whilst treatment with the D2 antagonist eticlopride was without effect (van Gaalen et al., 2006). Once again, the contrasting roles found of the DA receptors in the modulation of impulsive choice may be accounted for by the differing adjusting verses systematic delayed reward task being utilised across studies. The potential role of the D3 receptor in the modulation of delay aversion has additionally been explored. At the lowest dose tested activation of this receptor by the agonist 7-OH-DPAT led to an increased choice of the immediate smaller reward (van den Bergh et al., 2006).

The role of D1 and D2 receptors in the modulation of motoric impulsivity has furthermore been assessed. In direct contrast to the effects of the D1 receptor antagonist SCH23390 in an adjusting amount DD procedure, the antagonist has been shown to decrease premature responding in the 5CSRTT (Harrison et al., 1997; Koskinen and Sirvio 2000; Hahn, Shoaib and Stolerman, 2002; van Gaalen et al., 2006) while activation of the receptor following high doses the D1 agonist SKF 38393 increased premature responding (Pezze, Dalley and Robbins, 2007). Furthermore, pre-treatment with the D1 antagonist led to a reversal of increased premature responding on the task following treatment with cocaine, nicotine and amphetamine (van Gaalen et al., 2006) and moreover reduced heightened impulsivity expressed in 5-HT depleted animals (Harrison et al., 1997). The D1/D2 receptor antagonist fupenthixol, and D2 antagonist raclopride have also demonstrated evidence of a dose dependent decrease in premature responding (e.g. Koskinen and Sirvio 2000; Hahn et al., 2002). In a further study, whilst the D2 antagonist eticlopride had no effect on impulsive responding in the 5CSRTT, blockade of the D2 receptor did however significantly attenuate the heightened impulsive responding following treatment with cocaine, amphetamine and nicotine (van Gaalen et al., 2006).

In further models of behavioural inhibition, less consistent findings have been established. In the FCN blockade of the D2 receptor by the antipsychotic drug, haloperidol, significantly decreased the number of chain responses, consistent with an increase in impulsivity (Evenden 1998a). This finding however is inconsistent with a more recent study conducted by Liao and

Cheng (2005) who demonstrated that the D2 antagonist raclopride and D1 antagonist SCH23390, decreased significantly the frequency of non-reinforced burst responses in a comparable FCN procedure in rodents. In contrast, in the SST DA D1/D2 receptors appear to play little role in the stop process within the task, with administration of the antagonist fupenthixol displaying no effect on stop signal reaction time in rats (Eagle, Tuft, Goodchild and Robbins, 2007). The absence of effect is further supported in the human literature with no significant changes observed in stop signal reaction time following treatment with L-DOPA in children diagnosed with ADHD (Overtom et al., 2003). These findings reveal that DA may mediate more the ability to wait and withhold inappropriate premature responding, as measured in FCN and 5CSRRT, while playing no critical role in the ability to stop an already initiated response and switch to an alternative behaviour.

It is clear from the literature that modulation of DA neurotransmission affects both impulsive choice and disinhibition assessed in terms of the ability to wait and withhold inappropriate and premature responding. DA's neurochemical control over impulsivity furthermore appears to depend on the complex interaction with the serotonergic system (e.g. Harrision et al., 1997; Winstanley 2003a). At the DA receptor level particular importance of the role D1, and D2 and possibly D3 receptor has been highlighted. Supporting further that impulsivity is not a unitary construct, the D1 and D2 receptors furthermore appear to have distinctive and opposing roles in the modulation of impulsive choice and disinhibition.

DA is also a central neurotransmitter in drug addiction, and it is therefore highly plausible to suggest that DA plays a vital role in the neurochemical control of the relationship between impulsivity and addiction. As previously discussed, the ability of the majority of misused drugs, with the exception of benzodiazepines, to increase dopaminergic function in the mesolimbic system is considered crucial for their rewarding effects (Koob et al., 1994; DiChiara, 1999; Koob and Le Moal, 2001). Studies in the neurobiology of addiction have more recently suggested that DA increases alone fail to explain the complexity of addiction (Volkow et al., 2002), as dopamine levels are raised in both addicted and non-addicted individuals following administration of abused drugs (Volkow et al., 1997a). Rather it is the distinct increases in DA followed by DA decreases and the resultant interference of both the mesolimbic and mesocortical DA circuits that it regulates, that underlies the process of addiction (eg. Goldstein and Volkow 2002; Volkow et al., 2002). Low DA levels are associated with withdrawal (Lingford-Hughes and Nutt, 2003) and abstinence from the drug of abuse, with neuroimaging studies displaying evidence of for example of 50% less dopamine cell activity in detoxified cocaine addicts in comparison to controls (Volkow et al., 1997b).

The D1, D2, and D3 receptors that have been implicated in the modulation of impulsivity have furthermore demonstrated an important role in the addiction of a number of abused substances including cocaine, heroin, methamphetamine, nicotine and ethanol (e.g. Comings and Blum, 2000; Thanos et al., 2001; Volkow et al., 2002; Heidbreder et al., 2004; 2005; Nader et al., 2006; Schmidt, Anderson and Pierce, 2006; Schmidt and Pierce, 2006; Vengeliene et al., 2006;). More specifically, the activation of the D1 receptor in the NAc shell promotes reinstatement of cocaine seeking behaviour in rats (e.g. Schmidt et al., 2006; Schmidt and Pierce, 2006), while antagonism of this receptor by SCH23390 reduces the reinforcing properties of the stimulant (Barli and Pierce, 2005). Interestingly, activation of the D1 receptor as previously discussed increased premature responding in the 5CSRTT (Pezze et al., 2007), whilst blockade of the receptor attenuated the heightened impulsive responding following treatment with cocaine, amphetamine and nicotine (van Gaalen et al., 2006). It appears that activation of the D1 receptor is highly likely to mediate the lack of inhibitory control displayed by drug dependent individuals. This receptor may therefore prove to be a potential target for the treatment of the pathological impulsiveness observed in these individuals.

The D2 receptor has also received extensive interest within the field of drug addiction literature with this receptor found to be significantly reduced in the striatum in cocaine, opiate and alcohol abusers (Volkow et al., 1996; 1997b; 2001; Wang et al., 1997). Furthermore the downregulation of D2 receptors is an abnormality that has been found to persist for months following abstinence (e.g. Volkow et al., 1993; 1997b). D2 receptors have been implicated in both inhibitory control and sensitivity to delayed gratification, alterations in D2 receptor availability may therefore be a common abnormality relating impulsivity to drug dependence across a number of abused drugs. Moreover, evidence of the persistent dysfunction in impulsive control observed in addicts following long term abstinence (e.g. Petry, 2001; Kirby and Petry, 2004; Hoffman, 2006), in particular in cocaine abusers (e.g. Kirby and Petry, 2004; Heil et al., 2006), may be possibly mediated by the continual down regulation of D2 receptors that is observed in abusers.

A major limitation of these studies however is that they fail to determine whether, a dysfunction in dopamine and D2 receptors, linked to both the modulation of impulsive behaviour and addiction, is a secondary effect of chronic drug abuse possibly leading to the increase in impulsivity observed or is instead a predisposing factor to future drug abuse which would place already more impulsive individuals at a greater risk of drug addiction. Dalley and colleagues (2007) however have recently successfully connected impulsive responding, DA and addiction in rodents. Strong evidence was provided indicating that high impulsive rats, as assessed in 5CSRTT, displayed a significant reduction in D2 availability in NAc that rendered them more susceptible to increased rates of cocaine self administration (Dalley et al., 2007). The study supported earlier findings that both D2 receptor knockout mice and non-human primates with

low D2 receptor availability display higher rates of cocaine self administration (Caine et al., 2002; Nader et al., 2006). Human genetic studies have furthermore indicated evidence that variation in D2 receptor in polysubstances, opiate and nicotine abusers is linked to the D2 *Taq* IA1 and B1 allele (e.g. Smith et al., 1992; Comings and Blum, 2000; Xu et al., 2004; Gelernter et al., 2006). Taken together, these findings offer strong evidence of low D2 receptor availability being a biological trait that predisposes individuals more vulnerable to drug addiction, possibly through poor baseline levels of inhibitory control over behaviour. High levels of DA D2 receptors may therefore provide a defensive role against the abuse of drugs, through promotion of greater self control (Volkow et al., 2002). Indeed, delivery of D2 receptor gene into the NAc has been found to significantly reduce alcohol consumption in rats (Thanos et al., 2001). These findings should not be taken, however, as evidence that relationship between DA, impulsivity and addiction is unidirectional, as it is also possible that chronic drug exposure also further exacerbates an already dysfunctional DA system leading to the loss of control and compulsive intake that is observed in addicted individuals. Furthermore, it is yet to be established whether reduced D2 availability is a genetic trait that underlies heightened sensitivity to delayed gratification.

Finally, the D3 receptor is a receptor that is in addition receiving increased interest in addiction literature. The receptor which is mainly expressed in the mesolimbic brain, particularly the accumbens has been implicated in a number of addictive behaviours across drugs of abuse. For example, antagonism of the receptor appears to reduce nicotine induced conditioned place preference and locomotor activity (Pak et al., 2006), alcohol seeking behaviour (Vengeliene et al., 2006) and the self administration of cocaine in rodents (Xi et al., 2006). Although research is limited in terms of the role of this receptor in impulsivity recent evidence suggests that stimulation of the receptor by the agonist 7-OH-DPAT resulted in heightened impulsive choice in a DR paradigm (van den Bergh et al., 2006). D3 receptor antagonism may therefore constitute a promising novel pharmacological approach to treatment which may significantly reduce a number of addictive behaviours including intolerance to delayed reward.

1.4.2. Neuroanatomical Basis of Impulsivity

Research investigating the neuroanatomy underlying impulsivity has focused greatly upon areas of the both the ventral striatum (VS) and frontal cortex (FC), both regions of which have been implicated in addiction (e.g. Everitt et al., 1999; Jentsch and Taylor, 1999; Kalivas and Volkow, 2005).

1.4.2.1. The Role of the Ventral Striatum

The VS has been implicated in both the regulation of impulsive choice and inhibitory control. Research investigating the neurobiology of impulsive choice has demonstrated, using functional

magnetic resonance imaging (fMRI), that the activity of the VS is significantly elevated in healthy human subjects during the selection of the smaller, immediate reward during a delayed reward task (McClure et al., 2004). Hariri et al., (2006) have since extended these findings indicating that activity of the VS can furthermore discriminate between individuals of high and low impulsive choice, with hyper reactive VS circuitry associated with greater preference for the immediate reward. Authors concluded that abnormalities in the VS may contribute as a risk factor in the development of addiction.

More specific regions of VS have been associated with impulsive choice, most greatly the NAc. Cardinal and colleagues (2001) demonstrated that rats with lesions to the NAc core displayed heightened levels of discounting of delayed reward that could be attributed to an increase sensitivity to delay rather than magnitude of reward (Cardinal and Cheung, 2005). More recent evidence has furthermore suggested that the NAc role in impulsive choice is specific to the distinct subregion of the dorsolateral core with a lack effect on sensitivity to delayed reward shown following lesions to the ventromedial shell (Pothuizen et al., 2005). Lesions to the basolateral amygdala (BLA), a region strongly associated with NAc, had additionally increased impulsiveness of rats in DD paradigm (Winstanley et al., 2004a), suggesting that both the BLA and NAc may interact to modulate impulsive choice behavior. It is argued that both structures may be important in the representing and maintaining of the subjective value of reward across delay (Winstanley et al., 2004a). Failure in this ability in lesioned animals would therefore result in more impulsive choice.

Studies have moreover provided evidence that regions of the VS contribute to inhibitory control. Lesions to the NAc for example have demonstrated increased premature responding in a DRL procedure that was specified by later studies to be attributed to lesions the NAc core but not the shell (Reading and Dunnett, 1995; Pothuizen et al., 2005). Comparable findings of increased impulsive responding has additionally been shown in rats in the 5CSRTT (Christakou, Robbins and Everitt, 2004) but not the SST (Eagle and Robbins, 2003).

A further structure closely connected to the NAc whose role has additionally been explored in impulsivity is the subthalamic nucleus (STN) within the basal ganglia. Strong evidence for the role of the STN in inhibitory control has been provided with damage to the STN leading to a profound increase in premature anticipatory responding, as assessed in both the 5CSRTT (Baunez and Robbins, 1997) and DRL procedure (Uslaner and Robbins, 2006). In further support for the dissociated neurobiological modulation of varying forms of impulsivity, following lesions to this structure an opposite decrease in impulsive choice has however been shown (Winstanley et al., 2005; Uslaner and Robbins, 2006), suggesting that STN may differentially control subcomponents of impulsivity.

The VS, and more specifically the NAc, have long been implicated in the neurobiology of addiction. The region contributes and responds to both the anticipation and receipt of reward related stimuli, with hyperactivity of the region associated with a range of addictive behaviours including drug seeking, craving and reactivity to drug related cues (Robbins and Everitt, 1996; Everitt et al., 1999; Kalivas and Volkow, 2005). The region is recipient of both DA and 5-HT neurons and is a major terminal area of the mesolimbic DA system. The ability of drugs of abuse to stimulate the release of dopamine in the NAc is considered one of the most vital neurobiological process underlying addiction (Robinson and Becker, 1986; Kalivas and Stewart, 1991). The release of DA in this brain region is critical in the mediation of positive reward (Wise, 1990; Koob, 1992; Self and Nestler, 1995) relapse to drug seeking behaviour (Di Ciano et al., 1996; Shaham and Stewart, 1996; Self 1997) and psychostimulant drug craving (e.g. Robinson and Berridge, 1993) and motor effects (e.g. Berridge and Robinson, 1998; Salome et al., 2005).

The role of the VS in the regulation of range of addictive behaviour makes it a prime neuroanatomical location that may mediate the heightened impulsivity associated with drug dependent individuals. More direct evidence that NAc may be a candidate structure in the modulation of the relationship between addiction and impulsivity has been provided by a recent study of Pattij and colleagues (2007). Amphetamine induced disinhibited behaviour in a 5CSRTT was successfully blocked by the bilateral administration of the D2 antagonist eticlopride in NAc core but not the shell. Once again the importance of the D2 receptor in the NAc in the modulation of inhibitory control in addiction has been highlighted.

1.4.2.2. The Role of the Frontal Cortex

It is well established that prefrontal cortical mechanisms are heavily involved in impulse control, the subregions of which however may be differentially involved in different components of the behaviour (e.g. Aron et al., 2004). Humans with focal lesions to the medial prefrontal cortex (mPFC) have displayed a significantly greater inability to withhold responding on a go/no-go task (Picton et al., 2007), a finding much earlier demonstrated in monkeys (Iversen and Mishkin, 1970). Damage to the anterior ACC, prelimbic (PLC), infralimbic cortex (ILC) in rats, furthermore increased perseverative and premature responding, indicative of disinhibition, in the 5CSRTT (Muir et al., 1996; Chudasama and Muir, 2001, Passetti et al., 2002; 2003; Christiakou et al., 2004; Chudasama et al., 2004). Dalley et al., (2002), have in addition, highlighted the importance of 5-HT in the PFC in the modulation of baseline inhibitory responding. Rats exhibiting greater levels of premature responding displayed heightened extracellular levels of 5-HT in this region.

The OPFC has additionally been implicated in disinhibited responding. Lesions to this area of the PFC has led to augmented preservative responding in rats in the 5CSRTT (Chudasama et al., 2003), whilst in monkeys an increase in commission errors was observed during performance on the go/no-go task (Iversen and Mishkin, 1970; Butter et al., 1973).

In contrast, the ACC and mPFC appear to play little role in the regulation of impulsive choice (Cardinal et al., 2001). Selective excitotoxic lesions to both regions failed to affect choice that was indicative of alterations in sensitivity to delayed gratification, suggesting that these subregions of the prefrontal cortex contribute more to inhibitory control rather than impulsive choice. A further subregion of PFC, the OPFC, does in contrast, appear to contribute to impulsive choice. In rats lesions to the OPFC has led to both an increase (Kheramin et al., 2002; 2004; Mobini et al., 2002) and decrease (Winstanley et al., 2004) in the choice of the smaller immediate reward in systematic delayed reward paradigms. Whilst discrepancies in findings can be attributed to procedural differences across studies such as the time point of animal lesioning, quantitative analysis of choice behaviour has demonstrated that alterations in impulsivity can be attributed to changes in sensitivity of both magnitude of reward and delay as a result of damage to the OPFC (Kheramin et al., 2002; 2004).

As previously highlighted dysfunction within the regions of the frontal cortex is in addition considered a key neurological mechanism underlying addiction which may result in the associated loss of self control (e.g. Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004). For example, imaging studies have shown evidence of reduced gray matter volumes and densities within the region in polysubstance, cocaine, methamphetamine, alcohol and nicotine abusers (e.g. Pfefferbaum et al., 1997; Liu et al., 1998; Franklin et al., 2002; Brody et al., 2004). Furthermore, drug intoxicification, bingeing and craving are associated with activation of orbitofrontal and anterior cingulate cortices across addicts of varying drugs of abuse, whilst hypoactivation of these subregions are associated with long term withdrawal believed to be related the downregulation of D2 receptors (Brody et al., 2002; Goldstein and Volkow 2002; Rose et al., 2003; Neuhaus et al., 2006). More recently, greater hypoactivation in both the dorsolateral prefrontal cortex and parietal cortex has furthermore predicted relapse in methamphetamine users (Paulus et al., 2007).

Further evidence of impaired functioning of the OPFC in drug dependent individuals has additionally been provided using neuropsychological measures on which high performance is dependent on the normal functioning of this PFC region. An example of such a measure is the Bechara's Gambling task which assesses sensitivity to future outcomes of decisions. Significant impairments in performance have been observed in abusers of cocaine, methamphetamine and alcohol relative to control participants, with a high percentage of abuser's performance

moreover being found to be within the range of subjects with bilateral lesions to the OPFC (Bechara et al., 2001; Bechara and Damasio, 2002). In a further study, a causal relationship has been indicated between performance on this task and length of chronic abuse. Poorer performance on the task (indicative of a greater dysfunction of OPFC) was found to be positively correlated with years of amphetamine abuse (Rogers et al., 1999).

In summary, there is a growing body of evidence that both regions of the ventral striatum and frontal cortex are involved in the regulation of impulsive behaviour. Subregions however, appear to be differentially associated with impulsive choice and inhibitory control, with the NAc, and possibly the OPFC, appearing to be the only structures that control comparably both forms of impulsivity. The ACC and mPFC appear to be only strongly associated with inhibitory control while evidence of differential role of the STN in the control of both impulsive choice and disinhibition has been demonstrated. These brain regions are considered central to neurobiology underlying addiction with evidence of chronic drug exposure furthermore being associated with neural adaptations within these regions (Koob et al., 1998; Goldstein and Volkow, 2002). Abnormalities within these regions are highly likely, therefore, to underlie the heightened impulsivity associated with addiction. A causal association between these neurological mechanisms and impulsivity and or addiction however, cannot be corroborated from the majority of studies, leaving the direction of the relationship between these variables to be yet fully determined. It is possible that the neurobiological changes associated with drug abuse in these regions (e.g. Koob et al., 1998) may lead to a loss of control over drug intake and insensitivity to delayed consequences resulting in recurrent drug seeking, taking, and relapsing behaviour. Instead, these neurobiological abnormalities may reflect a vulnerability to drug abuse, a theory recently supported by Dalley and co-workers (2007). It is essential that future research continues in the attempt to disentangle this relationship.

1.5. OVERALL SUMMARY AND FUTURE DIRECTIONS

Impulsivity is a multifactorial concept, which in addition to being an important aspect of human personality and behaviour, in its pathological form is a symptom in a number of psychiatric disorders including substance abuse. Extensive research has demonstrated that heightened impulsivity, both in terms of an increase in sensitivity to delayed gratification and loss of inhibitory control, is strongly associated with drug dependence across different pharmacological classes of drugs of abuse suggesting that both subcomponents of impulsivity are key aspects of addiction.

Despite the continued growing body of evidence of the relationship between drug addiction and impulsivity an understanding of the complex nature of this association remains to be fully determined. As highlighted across the reviewed research one of the greatest limitations of the

majority of studies is that their cross sectional design does not permit inferences to be made regarding the causality or temporality of the relationship. Consistency of research demonstrating dose dependent type relations between patterns of abuse and impulsivity provides strong evidence that the association may be causal (e.g. Vuchinich and Simpson, 1998; Yakir et al., 2007). It is therefore possible that heightened impulsivity may be a risk factor that contributes to the establishment and maintenance of drug addiction or is instead a consequence of chronic drug abuse. The relationship between impulsivity and drug addiction may also be reciprocal; whilst trait impulsivity may be involved in the initiation of drug taking, with continued drug exposure the resultant neuroadaptions may cause further dysfunction in inhibitory control and decision making contributing to the maintenance of drug seeking and taking behaviour. From reviewing the current literature it is arguable that potential evidence has been provided for each of these directions, although currently no valid conclusions can as yet be made.

Research that has utilised self report personality measures (e.g. BIS) to discriminate between drug users and non-users support the theory that individuals with a more impulsive personality trait are at greater risk of developing addiction (e.g. Allen et al., 1998; Swann et al., 2004). This interpretation is based upon the premise that these measures are typically considered to assess long term stable behavioural traits. Unfortunately however, the abusers in the majority of such studies were not abstinent from the drug of abuse at the time of testing (eg. Moeller et al., 2002). Consideration therefore must be given to the possibility that it may have been the acute effects of the drug, or being in a state of withdrawal which led to higher scores of impulsivity on these measures being reported.

Research that has instead utilised behavioural tasks, argued to be sensitive to more subtle state changes in impulsiveness, have provided evidence that impulsivity may be a consequence of drug abuse. Administration of drugs of abuse have led to significant changes in levels of impulsivity, the effects of which have been shown to be dependent on a range of factors including the subcomponent of impulsivity being tested, dose level, length of drug exposure and basal level of impulsivity. Across stimulant drugs such as cocaine, nicotine and amphetamine, evidence of potential positive effects on impulsive behaviour have been shown, an effect however specific to low moderate doses and in individuals demonstrating heightened impulsivity at baseline. Unfortunately, a limited amount of research has assessed the effects of drug regimes more comparable to the pattern of drug use in dependent individuals. Those conducted however suggest that higher more chronic dosages may lead to the poorer inhibitory control and heightened impulsive choice observed in the drug dependent population (e.g. Richards et al., 1999a; Jentsch et al., 2002; Dallery and Locey, 2005).

Evidence has been additionally provided suggesting that initial drug deprivation may lead to the augmentation of impulsivity, at least in the case of impulsive choice (e.g. Field et al., 2006). Research has furthermore suggested that perhaps a bi-phasic relationship between drug abstinence and impulsive choice may exist. Initial drug deprivation may increase sensitivity to delayed gratification while longer term abstinence is associated with a reduction in levels of impulsivity (e.g. Bickel et al., 1999; Petry, 2001). It is yet to be elucidated conclusively whether impulsivity is indeed a reversible effect of dependence or, conversely, whether lower rates of impulsive responding may enable individuals to successfully remain abstinent (Bickel and Marsch 2001). The latter of these theories has been recently corroborated by findings suggesting that individuals with greater levels of DD are at greater risk of future relapse (Krishnan-Sarin et al., 2006). Long term abstinence, has in contrast, been associated with persistent impairments in inhibitory control relative to never abusers (e.g. Gourdriaan et al., 2005). These findings however were demonstrated in models primarily adopted to assess attention and no direct comparison of current and long term abstinent abusers were made. Despite this, if indeed chronic drug exposure induces impulsivity, then in terms of inhibitory control evidence suggests that these effects may persist for longer following abstinence. In contrast, impairments in behavioural control could have predated drug exposure and constitute instead a vulnerability for developing addiction.

Of the limited prospective human research that has been conducted support has been provided that both intolerance to delayed gratification and disinhibition are salient predictors of future substance use and possibly dependence (e.g. Tarter et al., 2003). Having no knowledge regarding the long term stability of performance on behavioural tasks of impulsivity, however, makes valid interpretation of such research currently difficult. On the contrary, more promising support for the role of impulsivity as a risk factor in the development of drug dependence has been provided from animal research indicating that highly impulsive animals self administer greater levels of cocaine and consume larger amounts of ethanol (e.g. Poulos et al., 1995; Dalley et al., 2007).

Research investigating the neural processes underlying impulsivity suggests that the adaptations of the serotonergic and dopaminergic systems associated with chronic drug abuse may mediate the heightened impulsivity in this population. The neurochemical processes underlying the relationship are, however, complex involving interactions of both systems and a dissociation of the role of receptor subtypes in the mediation of both impulsive choice and disinhibition. The 5-HT_{1A} and D₂ receptors in particular, appear to play a crucial role, and provide promising targets for future pharmacological treatments of impulsivity in drug dependence (Liu et al., 2004; Dalley et al., 2007). Strong evidence furthermore suggests that abnormalities within regions of the VS and PFC, considered central to the neurobiology of addiction, are involved in the

regulation of the loss of control and sensitivity to delayed gratification in abusers (e.g. Jentsch and Taylor, 1999; Cardinal et al., 2001). Subregions of these brain areas moreover, appear to be differentially associated with each of the subcomponents of impulsivity, providing further support of their dissociation at neurobiological level (e.g. Uslaner and Robinson, 2006). Yet to be fully elucidated is whether the dysfunctions in neurobiology reflect a biological trait vulnerability leading to heightened impulsivity and the development of drug dependence, or conversely abnormalities arise from chronic drug exposure promoting disinhibited behaviour and impulsive choice resulting in the maintenance of drug use. Presently, support for each of these directions has been provided (e.g. Levy et al., 1993; Dalley et al., 2007).

It is important that future research adopt more prospective designs that allow causal inferences to be made regarding the relationship between impulsivity and drug abuse. Research that assesses, within subjects, subcomponents of impulsivity both prior to and during chronic exposure, will enable questions to be answered regarding the extent to which impulsivity constitutes a risk factor to drug abuse and furthermore the possibility that drug exposure augments impulsiveness. Furthermore, exploring levels of impulsivity both at the point of initial withdrawal through to longer term abstinence will additionally further elucidate the potential role of impulsivity in relapse and determine whether drug induced changes in impulsivity are a permanent effect. The implications of such research in the field of addiction are substantial. If impulsivity is discovered to be a risk factor in the development of substance abuse, then those vulnerable may be identified and prevention strategies put in place. If it is determined that substance abuse enhances impulsiveness, which may play a role in the maintenance and relapse of the disorder, then behavioural and pharmacological treatments targeting impulsivity may lead to effective interventions for drug dependence (Nestler, 2003). In terms of human research, only long term longitudinal research will enable definitive answers to begin to emerge regarding the causality of the association between impulsivity and drug dependence. The importance of future preclinical research to additionally attempt to establish the nature of the relationship is essential. Such research will vitally provide the opportunity to further explore the neurobiological processes underlying the complex relationship between impulsivity and addiction.

1.6. THESIS AIMS AND OBJECTIVES

Despite the establishment of the association between impulsivity and drug addiction, research directly examining the nature of this relationship is limited. Research utilising animal models of impulsivity provides the opportunity to both disentangle the causality of the relationship and explore further the biological mechanisms underlying this association (Olmstead, 2006). It does this by overcoming some of the disadvantages associated with human longitudinal research such as attrition, time and costs. Additionally the precision of preclinical experimentation in animal

models has critical advantages. Firstly, such research enables the controlled manipulation of drug exposure. Secondly, animal models of impulsivity overcome limitations associated with the complex patterns of polysubstance abuse and sometimes co-existing psychopathology in human drug abusers; factors that make valid conclusions regarding the relationship between addiction and substance dependence often difficult. The main aim of the present thesis was to explore further fundamental aspects of the relationship between impulsivity and addiction through the adoption of animal paradigms of impulsive choice and inhibitory control.

1.6.1. Nicotine

The drug selected to explore the relationship between impulsivity and drug dependence in the present thesis is the psychoactive drug nicotine. Nicotine is the main addictive component of tobacco that is critical for the drive and maintenance of smoking behaviour (Kumar and Lader, 1981; Stepeny, 1982; Stoleran and Jarvis, 1995; Balfour et al., 2000). Although nicotine is not classed as one of the 'harder' drugs of abuse, cigarette smoking is a prototypic drug addictive disorder sharing common features at both the behavioural and neurobiological level. Comparable to other drugs of abuse, nicotine elicits drug seeking behaviour under controlled laboratory conditions, supported by the self administration and conditioned place preference observed in animals (e.g. Corrigall, 1999; Di Chiara, 2000). The drug's ability to indirectly enhance DA release in the NAc, is believed to be central to the psychostimulants rewarding and motivational properties, a neurobiological pathway shared by the majority of abused substances (e.g. Stoleran, 1997; Piciotto, 1998; Di Chiara, 2000). Chronic nicotine cessation is furthermore associated with a withdrawal syndrome that has been identified in both humans (Hughes et al., 1991; Hughes 2007a; 2007b) and animals (e.g. Malin et al., 1992; Epping-Jordan, Watkins, Koob and Markou., 1998; Harrison, Liem and Markou, 2001) that manifests both somatic and affective symptoms. The fact that classic features of addiction are associated with nicotine dependence is of particular importance in that findings of the present thesis may elucidate also the complexity of the association between impulsivity and drug abuse in other addictive disorders.

Focusing on nicotine in an attempt to further current understanding of the relationship between impulsivity and dependence is well warranted for a number of reasons. Firstly, tobacco smoking is currently the world's most prevalent substance dependence with research estimating that currently a third of the population are smokers. (Centres for Disease Control and Prevention, 1999; WHO, 2005). Secondly, cigarette smoking is a major health problem, representing the largest preventable risk factor for premature death in the western world (Peto et al., 1996; Bergen and Caporaso, 1999; Leshner, 2000). It is a key cause of cardiac, vascular and pulmonary diseases, as well as a variety of cancers leading researchers to estimate that approximately 6.5 million people worldwide will perish per annum from smoking related

diseases by 2015 (Mathers and Loncar, 2006). Thirdly, nicotine addiction is a chronic relapsing disorder. Despite the recent advances in the current pharmacological and behavioural treatment for nicotine dependence, a high percentage of individuals who express a desire to quit smoking are continuing to fail in their ability to successfully abstain (Baer and Marlatt, 1991; Balfour and Fagerstrom, 1996; Hughes et al., 1999; Ebbert et al., 2007; Mitrouska, Bouloukaki and Siafakas, 2007).

The substantial associated human and economic harm of smoking and lack of effective current interventions for nicotine dependence therefore warrants continued research investigating both the psychological and biological mechanisms of nicotine addiction in the hope of informing prevention and treatment efforts for the disorder. Surprisingly, despite the consistent heightened impulsivity associated with heavy smoking, limited research in the animal literature has explored the relationship between impulsivity and nicotine addiction, focusing instead on the effects of illicit agents. Only one study has reported the effects of nicotine in an animal paradigm of DD (Dallery and Locey 2005), whilst the effects on inhibitory control have been limited to models primarily used to assess sustained and divided attention (e.g. Blondel et al., 1999). The lack of preclinical research coupled with the huge societal costs of nicotine addiction therefore makes the research of this thesis both novel and an essential avenue of investigation.

The principal aim of this thesis was to therefore further current understanding of the relationship between impulsivity and nicotine addiction through a series of preclinical studies. As previously argued, the heightened impulsivity observed in smokers relative to non-smokers could be attributed to either a pre-existing difference or to the direct effects of nicotine (e.g. Bickel et al., 1999; Spinella, 2002). This thesis is primarily concerned with exploring the latter of these theories. If augmented impulsivity is induced by nicotine, this could be due to either the direct acute effects of the drug, the long term exposure to nicotine, or to the effects of nicotine withdrawal, each of which could play a role in the maintenance and relapse of the disorder. Each of these possibilities was explored in Lister Hooded rats in two operant paradigms, each measuring a distinct component of impulsivity. A systematic delayed reward paradigm was adopted to assess impulsive choice (Evenden and Ryan, 1996; Cardinal et al., 2000), whilst the symmetrically reinforced conditioned visual discrimination go/no-go task (e.g. Harrison et al., 1999) was utilised to measure inhibitory control. Impulsive choice and inhibitory control have demonstrated often dissociable relationships with addiction at both the behavioural and neurobiological level (e.g. Reynolds et al., 2006a; Talpos et al., 2006); it was therefore essential to adopt multiple measures of impulsivity to allow a more accurate understanding of the effects of nicotine on impulsivity to be gained.

Prior to drug experimentation a series of preliminary control studies were first conducted. These studies enabled the assessment of the suitability of both models for the investigation of acute and long term nicotine administration and nicotine withdrawal.

The main objectives of the present thesis were more specifically:

- i) To assess the suitability of the symmetrically reinforced go/no-go conditioned visual discrimination task (Experiment 1) and the delayed reward task (Experiment 5) as models to explore the relationship between nicotine addiction and impulsivity.
- ii) To assess the acute effects of nicotine in animals with no prior exposure to the drug on inhibitory control (Experiment 2) and impulsive choice (Experiment 6). Furthermore, in order to verify that the effects of nicotine were mediated by central nicotinic receptors, attempts were made to block effects with the nicotinic receptor antagonist mecamylamine.
- iii) To assess, adopting a longitudinal design, the effects of chronic nicotine exposure and initial and long term nicotine withdrawal on inhibitory control (Experiment 3) and impulsive choice (Experiment 7). Such research designs permitted investigation of a) more comparable patterns of drug use of dependent smokers on impulsivity, b) of the potential role of impulsivity in relapse during withdrawal and c) the permanence of nicotine induced effects on impulsivity.
- iv) To assess changes in responsivity to nicotine following a period of sustained abstinence. This was achieved through assessment of the effects of a series of acute nicotine challenges in animals previously exposed to chronic nicotine and nicotine withdrawal on inhibitory control (Experiment 4) and impulsive choice (Experiment 8). Such research enabled further exploration of the role of impulsivity in relapse.
- v) Finally, in view of the clear identification of high versus low trait impulsive animals, as assessed by the model of delayed reward, preliminary exploration was made of the differences in response to chronic nicotine and withdrawal based upon basal levels of impulsive choice (Experiment 7). Furthermore, exploration of differences between high and low impulsive animals in response to acute nicotine challenges following a period of abstinence were additionally assessed (Experiment 8).

CHAPTER 2

General methodology

The following methodology sections refer to standard procedures that apply to the majority of studies in this thesis. Any variation in the standard methodology will be highlighted in the reports of individual experiments.

2.1. ETHICS

All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986. Prior to conducting experimental procedures, the experimenter passed four Home Office-accredited training courses (modules 1 - 4) at the University of Leeds, leading to the granting of a Personal Licence (PIL 40/7373). Experiments 1-8 were conducted under the Project Licence of Dr. Amanda Harrison (PPL 40/2711). The health of all animals was monitored daily. Animals displaying any signs of ill health or distress were immediately removed from study.

2.2. SUBJECTS

The subjects in all studies were adult male Lister hooded rats (Charles River, UK) weighing approximately 300-320g at the start of testing. Animals were housed in pairs (cage size; 46 X 26.5 X 26cm) on arrival at the laboratory. To induce motivation for food reward, all subjects undertaking operant testing were food restricted in order to maintain them at 85% of their free feeding adult body weights (BW) throughout the testing period. Water was available ad libitum in home cages. Feeding occurred at the end of each experimental day, during which, animals were separated.

Animals were maintained under a 12 hour light/dark (LD) cycle (lights on at 0700h; lights off at 1900h) at a controlled environmental temperature of $21.5^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and relative humidity of $50\% \pm 10$. Air changes occurred twenty times per hour. During the light phase, the holding room was illuminated by artificial lighting (approximately 193 lux). On the rare occasion when animals were tested during the dark phase, this was conducted under low intensity red light (approximately 2 lux).

2.3. DRUGS

All drug doses were selected on the basis of previous literature reporting behavioural effects

(e.g. Turek, Kang, Campbell and Sullivan.,1995; Hildebrand et al., 1997; Bizot et al., 1998; Epping-Jordan et al., 1998; Blondel et al., 2000; Popke, et al., 2000a; Stoleran et al., 2000; Watkins, Stinus, Koob and Markou, 2000; Harrison et al., 2001; Dallery and Locey, 2005). All drug solutions were prepared on the day of administration.

2.3.1. Nicotinic Acetylcholine Receptor Agonist

(-)-Nicotine Hydrogen tartrate salt ((-)-1-Methyl-2-(3-pyridyl)pyrrolidine(-)-Nicotine(+)-bitartrate salt) was purchased from Sigma-Aldrich Inc (Poole, UK). The compound was dissolved in 0.9% saline and the pH adjusted to approximately 6, using 0.1 M sodium hydroxide. Nicotine was administered s.c. in a volume of 1ml/kg at doses 0.125, 0.25, 0.5 and 1.0mg/kg. Drug dosage was calculated as free base. An active measure of 0.3508mg nicotine was present in 1mg of nicotine hydrogen tartrate salt.

Acute nicotine treatments were administered 10 minutes prior to behavioural testing. The injection test-interval was selected on the basis that nicotine is both rapidly absorbed and has a short half life of 60-90 minutes following systemic injection (Schechter and Jellinek, 1975).

Nicotine was administered chronically through subcutaneous osmotic mini pumps (length: 5.1cm; diameter: 1.4cm; weight: 5.1g) (Model 2ML1; Alzet, Charles River, UK). The nicotine concentration was adjusted to compensate for differences in BW, resulting in a delivery of a dose of 3.16mg/kg/day for a duration of seven days. This daily dose maintains plasma levels of nicotine at approximately $\sim 44\text{ng ml}^{-1}$ - a level which is similar to smokers who consume 30 cigarettes per day (Murrin, Ferrer, Zeng and Hayley, 1987; Benowitz 1988). The drug solution was released at a rate of 10 $\mu\text{l/hr}$ across a seven-day period. Due to the start up gradient of pumps, drug release began approximately six hours following implantation.

2.3.2. Nicotinic Acetylcholine Receptor Antagonist

Mecamylamine hydrochloride (2-Methylamino isocamphane hydrochloride Inversine N,2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride) was purchased from Sigma-Aldrich. This centrally acting non-competitive receptor antagonist was dissolved in 0.9% saline and administered s.c. in a volume of 1ml/kg at doses 0.1, 0.3, and 1.0mg/kg. Doses were calculated as free base. In 1mg of mecamylamine hydrochloride, 0.82mg of mecamylamine was present. On the basis of past literature data and the rapid diffusion of mecamylamine across the blood-brain barrier (e.g. Debruyne et al., 2003), the nicotinic antagonist was administered 20 minutes prior to testing.

2.4. SURGERY

Alzet osmotic mini pumps were surgically implanted to deliver nicotine chronically. Osmotic mini pumps are a commonly used preclinical research tool for the chronic delivery of addictive agents such as nicotine (e.g. Epping-Jordan et al; 1998; Harrison et al., 2001). Unlike repeated injections, osmotic pumps permit the continuous delivery of drugs at controlled rates. This ensures the maintenance of constant plasma drug levels throughout the study, which is vital when attempting to mimic the nicotine intake of smokers. Evidence suggests that smokers attempt to maintain a constant blood level of nicotine, through varying both the quantity of cigarettes consumed and actual smoking behaviour (e.g. Chait and Griffiths, 1982; McMorrow and Foxx 1983; Chait, Ross and Griffiths, 1985). Mini pump implantation has the further advantage of eliminating the stress associated with repeated handling and injections, which may impact on results.

2.4.1. Subcutaneous Implantation

Pumps were implanted under general anaesthesia. Induction of anaesthesia was achieved through the delivery of a concentration of 4% isoflurane into an induction chamber. Once anaesthetised, animals were maintained on a mixture of 1.5-3% isoflurane and oxygen throughout the surgical procedure. The pumps were implanted subcutaneously, to the right of the Thoracic vertebra. A mid-scapular incision of approximately 2cm in length was made, and through a blunt dissection, a secure pocket was made for the pump. The filled pump was implanted and the incision closed with 9mm steel wound clips (Vet-Tech, UK). The topical antibiotic Cicatrin (Vet-Tech, UK) was applied to the wound. All pumps were weighed prior to implantation.

2.4.2. Subcutaneous Explantation

Animals were anaesthetised as above. Surgical explantation of the pump involved making a scapular incision across the previous skin opening. The pump was removed and during this procedure the incision closed with Vetbond surgical adhesive (Vet-Tech, UK). Following removal, in order to verify that the drug was delivered, pumps were re-weighed and the residual volume in all pumps recorded.

During both surgical procedures, animals were normally under anaesthesia for no longer than a duration of 15 minutes. Both the adoption of inhalation gases and the short surgical procedure meant that the recovery time for animals was rapid, thus having limited impact on behavioural test procedures.

2.5. OPERANT TESTING

Two operant paradigms were adopted to measure impulsivity. The symmetrically reinforced go/no-go conditional visual discrimination task was utilised to measure inhibitory control (Harrison et al., 1999), and the delayed reward paradigm was adopted to assess levels of impulsive choice (Evenden and Ryan, 1996; Cardinal et al., 2000). Each of these tasks were conducted in operant test chambers.

2.5.1. Apparatus

Two sets of four operant test chambers were used for behavioural paradigms (dimensions 30.5 X 24.1 X 29.2cm and 30.5 X 24.1 X 21cm; Med Associates Inc., USA). Each aluminium chamber was enclosed within a soundproof wooden box, and this was fitted with a ventilator fan to provide air circulation. The chamber was illuminated by a 2.8 W stimulus house light and fitted with two retractable levers on the front wall of the chamber. Above each lever was a 2.8 W stimulus light. Located between the centre of the two levers was a food magazine to which 45mg sucrose pellets (Noyes pellets, Sandown Scientific, UK) were delivered from a pellet dispenser. The magazine was illuminated by a white LED and head entries into the magazine were detected by a horizontal infrared photobeam across the entrance. All programs controlling the apparatus and collecting the data were written by the experimenter using Med-PC software running on a Pentium 3 Processor computer.

2.5.2. Operant Testing Procedure

Animals for all studies were habituated to laboratory conditions and handled for two weeks prior to training. During the first week, animals were given free access to food and water. Following this a food deprivation schedule of 18.6g/per day (inclusive of food reward gained in the operant chamber) was implemented in order to maintain animals at 85% of their free feeding adult bodyweights. Bodyweights were recorded daily (approximately 0800h), beginning one week before training, and continuing throughout the study.

Each experimental day, animals were transported in their home cages from the holding room to the test laboratory and placed in the operant chambers. The test chamber and time of testing remained constant throughout the study for each animal. Between animals, fresh wood shavings were placed on the floor of the operant chamber. Once animals had finished testing, they were returned to their holding room in their home cage. Animals, unless otherwise stated, were tested once a day during the light phase of their LD cycle. Testing took place between 0800h and 1800h. Feeding took place at the end of each experimental day, during which time, animals were separated to ensure they consumed the calculated amount of normal rat chow. One animal from each pair was removed from the home cage and placed in an adjacent feeding cage;

separating the animals during feeding aided the maintenance of body weights. During feeding, animals were separated no longer than one hour per day.

2.6. NICOTINE ABSTINENCE SYNDROME

A nicotine withdrawal syndrome has been observed in humans following the cessation of smoking (e.g. Hughes and Hatsukami, 1986; Hughes et al., 1991) and in rats following both the termination of chronic nicotine treatment and the administration of the centrally acting antagonist mecamylamine (Malin, 1992; Malin et al., 1994; Hildebrand et al., 1997; Epping-Jordan et al., 1998; Harrison et al., 2001). In order to assess nicotine withdrawal, the animal model of nicotine abstinence, which is based upon the spontaneous somatic signs shown in animals in withdrawal, was adopted (Malin, 1992). Using a nicotine abstinence scale, developed by Malin (1992), the intensity of withdrawal can be quantified by assessing the frequency of somatic signs shown.

2.6.1. Apparatus

During the observation of somatic withdrawal signs, each animal was placed in a glass observation tank (dimensions 40.5 X 37 X 11 cm). The size enabled animals to express a range of abstinence signs, but was also limited in an attempt to discourage exploratory behaviour. The same tank was used for all animals. Behaviours were recorded by a video camera (JVC TK-128OE) positioned horizontally in front of the observation tank. The camera was relayed to a monitor (TM-1500PS) in an adjacent laboratory.

2.6.2. Assessment of Somatic Withdrawal Signs Procedure

During the observation of somatic signs, animals were transferred in their home cages to the observation laboratory. Both saline and nicotine treated rats were observed for somatic withdrawal signs. All animals were habituated to both the test room and observation chamber prior to testing. During habituation, animals were placed in the observation chamber for 10 minutes on two consecutive days prior to the first baseline assessment of somatic signs. During this period, the experimenter remained in the observation test laboratory to accustom animals to the presence of the experimenter.

Animals were assessed individually by being placed in a glass observation chamber and the frequency of behaviours was recorded by the experimenter for a period of 10 minutes. The experimenter remained seated at a distance of approximately 1.5 metres away from the observation chamber. In addition, the behaviours were recorded by a video camera. This enabled, when necessary, to verify observations made. The same tank was used for all animals. The floor of the tank was covered with wood shavings. Between animals, the observation tank was thoroughly cleaned and fresh wood shavings provided. The number of somatic signs, including gasps, writhes, body shakes, head shakes, chews, teeth chattering, cheek tremors, paw tremors, genital grooming, foot licks, yawns, ptosis and scratches were recorded. A description of these behaviours is summarised in Table 2.1. For convenience in statistical analysis, gasps and writhes were aggregated, as were teeth chatters and chews, and all categories of shakes and tremors. The remaining behavioural categories were combined to form a category of “miscellaneous somatic signs”.

Somatic signs were assessed the day before pump implantation, the final day of chronic drug infusion, and during the week following the termination of drug treatment. For specific timings of assessment during withdrawal see individual study methodologies. For the majority of observations, somatic symptoms were recorded immediately following operant behavioural testing and under normal laboratory illumination during the light phase of their LD cycle. The exception to this was during the early onset of withdrawal, when somatic signs were also observed during the animals dark phase of their LD cycle. During this time, the test laboratory was illuminated under low intensity red light (approximately 0.01 lux).

2.7. STATISTICAL ANALYSIS

All statistical analyses were conducted using SPSS (Statistical Package for Social Sciences Version 14.0) and Microsoft Excel (Microsoft Office 2000)

Appropriate descriptive statistics were performed on all data and evaluated for homogeneity of variance, sphericity and assumptions of normality prior to analysis. Homogeneity of variance

was assessed by Levene's test (between-subject designs) and Mauchly's test of sphericity (within-subject designs). Normal distribution of scores was explored through both histograms and Shapiro-Wilks tests. Any data that violated these assumptions were subjected to the appropriate transformations. In the case of proportional data (e.g. percentage correct trials and percentage choice) data was subjected to arcsine transformations. All other dependent measures were subjected to either square root, log10 or inverse transformations (Tukey, 1977; Howell 1992; Tabchnick and Fidell, 2007). For detailed information on the statistical analyses performed refer to individual study methodologies.

Table 2.1: Behavioural somatic measures of nicotine abstinence syndrome

Behavioural Measure	Dependent Variables
Gasps	An accordion-like movement of the animal's chest along the long axis of the body.
Writhes	Contraction of the abdominal muscle wall in the form of the animal either pressing or flattening its abdomen against the chamber floor, or lifting or drawing its abdomen in (sometimes while arching its back).
Body Shake	A rapid, repetitive movement of the head, neck and trunk along the animal's central axis.
Head Shake	A rapid, repetitive movement of the head and neck along the animal's central axis.
Chew	Three or more consecutive opening and closing motions of the animal's mouth. Chewing is not recorded again unless there has been a distinct pause between episodes or unless 15 seconds of continuous chewing has passed.
Teeth Chatter	An audible clicking sound, made when the animal's teeth come together rapidly. Teeth chatter is recorded if it occurs simultaneously with chewing or cheek tremors.
Cheek Tremor	Obvious tremor occurring in the fleshy part of animal's cheek, almost directly below its eye. Cheek tremor is not recorded again unless there has been a distinct pause between episodes or unless 15 seconds of continuous tremors has passed.
Paw Tremor	Obvious quiver of the forepaw while lifted off the chamber floor. Not recorded if the animal begins grooming immediately after.
Foot Lick	Animal licks hind legs. Front legs are not included because they are commonly a part of grooming activity.
Genital Licks/ Seminal Ejaculation	Grooming movements of the head toward the genital region so that the head points between the hind legs. The top of the head is almost parallel to the chamber floor. Penis may be unsheathed and visible.
Scratches	Animal uses the hind leg to scratch anywhere on its body. Scratches on or close to healing wound area are not recorded.
Ptosis	At least one of the eyelids droop. Recorded when the eyelids narrow to a virtual slit for a longer duration than a normal eye blink. Not recorded again until one minute has passed.
Yawn	Animal opens its mouth, lowering its jaw and tensing muscles around the mouth area. Tongue curls downward into the jaw.
Overall Signs	Somatic signs accumulated across all categories.

Adapted from Malin et al., (1992).

CHAPTER 3

Validation of the symmetrically reinforced go/no-go conditional visual discrimination paradigm

3.1. INTRODUCTION

3.1.1. General Introduction

Disinhibition is one aspect of the multifactorial construct of impulsivity (Evenden, 1999), and has been defined as the inability to appropriately withhold or terminate thoughts and actions (Logan, Cowan and Davis, 1984). Disinhibition is often referred to as motoric impulsivity (Brunner and Hen, 1997), and as previously discussed in Chapter 1 a variety of paradigms have been devised in both the human and animal literature to assess this form of impulsivity.

One of the most commonly utilised paradigms, which has been successfully modelled in preclinical research for use with animals, is the symmetrically reinforced go/no-go visual discrimination task (Newman, 1987; Newman and Kosson, 1987; Fletcher, 1993). Unlike many other animal models argued to measure disinhibition, such as the DRL and 5CSRTT, this task was designed primarily to assess levels of motoric impulsivity. For this reason, the go/no-go task was the paradigm chosen to explore the relationship between inhibitory control and nicotine dependence in the present thesis. The rodent version of the inhibitory task has been described in detail previously (see section 1.3.2.1). In brief, to be food rewarded animals are required to learn to discriminate between two visual stimuli; one requiring active lever responding during Go trials, the second the requiring of withholding of a lever response during No-go trials. More impulsive animals display a greater difficulty in withholding responding during No-go trials (Fletcher, 1993). The task employed is symmetrically reinforced, therefore both discriminative stimuli represent the availability of reward and responding impulsively during No-go trials consequently leads to the *loss* of reinforcement. This task differs from asymmetrical paradigms where only active responding during Go trials is rewarded. The importance of utilising a symmetrically rewarded procedure is that it enables dysfunctions in inhibitory control to be dissociated from that of attenuated aversiveness to non-reward (Newman, 1987; Harrison et al., 1999).

A further advantage of the go/no-go paradigm is that in addition to accuracy of performance during Go and No-go trials, measurement of speed of responding and anticipatory behaviour during task performance can also be made. Although the primary measure of disinhibition in the

task is the inability to withhold responding during No-go trials, anticipatory early responding and inappropriate magazine entries are also arguably further indexes of impulsive behaviour (Harrison et al., 1999; Paine and Olmstead, 2004). Having an array of behavioural parameters on which the effects of drug manipulations can be observed allows a more accurate profile of the drug induced effects on disinhibition to be gained.

3.1.2. Stability of Disinhibition

A main objective of this thesis was to adopt chronic long-term research designs that afford causal interpretation of the relationship between inhibitory control and drug dependence. However, in order to assume that any changes in behaviour during chronic preclinical studies are attributable to the pharmacological manipulations conducted, verification is needed of the stability of behaviour in the absence of these manipulations. To date, information regarding the stability of impulsive behaviour is minimal, in particular in relation to levels of inhibitory control (Mitchell, 2004). Research has been conducted that suggests that the level of anticipatory responding in the 5CSRTT varies extensively across rats but is however a stable 'trait' that remains unchanged throughout adulthood (Robbins, 2002; Dalley et al., 2007). Utilising a comparable go/no-go task as in the present research, Harrison et al., (1999) furthermore demonstrated that the ability to withhold responding during No-go trials remained relatively stable in sham operated animals over a 20 day period. In contrast, evidence of a gradual increase in accuracy during Go trials was observed.

3.1.3. The Effects of Alterations in Primary Motivation on Disinhibition

When investigating the effects of drugs of abuse in operant models that utilise food reinforcers, it is crucial to demonstrate that the observed alterations in behaviour are not governed simply by drug induced changes in the value of reward. This is of particular importance when investigating the stimulant nicotine. It is well established that nicotine suppresses appetite whilst cessation of smoking or nicotine treatment can lead to hyperphagia and weight gain (Grunberg 1982; Grunberg, 1986; Grunberg, Bowen and Winders, 1986; Klesges et al., 1989; Miyata et al., 1999; Pomerleau et al., 2000; Zhang et al., 2001). To aid interpretation of drug induced behavioural changes in animal models of disinhibition, previous research has investigated alterations in primary motivation on task performance in control animals. The value or motivation for food reward can be manipulated through varying levels of deprivation, either through prefeeding prior to the test session or maintaining animals at varying proportions of their adult body weights through restricting or increasing of daily food intake (e.g. Skinner, 1938; Heyman and Monaghan, 1987; Rolls et al., 1995; Aoyama 2000). Utilising such methodology research has demonstrated that disinhibited responding, as assessed by the 5CSRTT, is significantly reduced following the decrease in motivation for food reward (Carli and Samanin, 1992; Harrison et al., 1997; Grottick and Higgins, 2000; Grottick and Higgins,

2002; Bizarro and Stolerman, 2003). Conversely, increasing motivation for food reward has been reported to enhance levels of anticipatory responding in the 5CSRTT (Bizarro and Stolerman, 2003). These findings suggest that caution should be implemented when interpreting drug effects on measures of disinhibition in the 5CSRTT, as they may be indicative of changes in motivation rather than impulsivity. It is currently unknown whether measures of disinhibition in the go/no-go task are additionally sensitive to alterations in levels of primary motivation.

3.1.4. Timing Behaviour

A further factor that has hindered the interpretation of the effects of drugs of abuse in animal models of disinhibition, such as the DRL, is that performance in such tasks is highly dependent on timing ability (Paule et al., 1999; Wiley et al., 2000). As timing mechanisms are known to be sensitive to a number of drugs of abuse, including nicotine (e.g. Carrasco et al., 1998), it is essential that performance in the go/no-go task is not dependent on time perception. Harrison et al., (1999) has previously argued that in contrast to other models of disinhibition, the need for timing behaviour is substantially reduced in the go/no-go paradigm, due to the presence of explicit extroceptive cues informing the animal when to and not to respond. This was not, however, explicitly investigated.

3.1.5. Objectives of Experiment 1

To determine the suitability of the symmetrically reinforced go/no-go conditional visual discrimination task for the investigation of the relationship between nicotine dependence and disinhibition, a series of preliminary studies were therefore conducted to assess:

- i) The stability of performance in the go/no-go task (Experiment 1A and 1B).
- ii) The effects of acute alterations in primary motivation on task performance (Experiment 1C).
- iii) The dependency of accuracy of performance on timing behaviour (Experiment 1D).

These objectives were achieved through conducting a series of preliminary studies in a control group of animals. The first objective was achieved through assessment of the stability of performance in the task across a three week period. As assessment of levels of inhibitory control during long term drug studies would, at stages, involve animals performing the task twice per test day, stability of task performance was also examined during a seven day period during which the number of daily test sessions was increased. The acute effects of alterations in primary motivation on task performance were then assessed by varying the level of motivation for food reward, through either prefeeding subjects immediately prior to the test session or acutely increasing levels of deprivation. Finally, to explore whether timing behaviour was necessary to accurately perform the task, the duration of stimulus presentation during no-go

trials was varied during a single test session. If the withholding of responding during no-go trials was not dependent on timing ability then such a manipulation would have no effect on levels of inhibitory control.

3.2. EXPERIMENT 1(A-D): VALIDATION OF THE SYMMETRICALLY REINFORCED GO/NO-GO CONDITIONAL VISUAL DISCRIMINATION TASK: PRELIMINARY CONTROL STUDIES

3.2.1. METHOD

3.2.1.1. Subjects

Subjects were 8 adult male Lister hooded rats (Charles River, UK) weighing 300-320g at the start of training. On arrival to the laboratory animals were housed in pairs (cage size; 46 X 26.5 X 26cm) and maintained under a 12 hour light/dark cycle (lights on at 0700h; lights off at 1900h) at a controlled environmental temperature of $21.5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity of $50\% \pm 5$. Animals were maintained at 85% of their free feeding adult body weights throughout the testing period. Water was available ad libitum in home cages and feeding occurred at the end of each experimental day. The same subjects were utilised for Experiments 1 (A-D). During testing however two subjects were removed from study due to illness. One subject was removed during Experiment 1A, a further subject during Experiment 1B. Due to failure to reach criterion performance until the fourth month of testing, a third subject only performed Experiments 1B-D. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986.

3.2.1.2. Apparatus

Four identical operant chambers were used (30.5 X 24.1 X 29.2cm Med Associates Inc., USA). See general methodology of Chapter 2 section 2.5.1. for a more detailed description of apparatus.

3.2.1.3. Behavioural Testing: Symmetrically Reinforced Go/No-go Conditional Visual Discrimination Task

3.2.1.3.1. Pre-training

Food deprived animals were initially magazine trained. During the first of these sessions animals were placed in an operant chamber with several sucrose pellets in an illuminated magazine. Continuous reinforcement (CRF) magazine training then followed. During a single training session the magazine light was illuminated, which signalled the availability of food reward. If the animal's head entered the illuminated magazine, a signal food pellet was delivered. Animals were then lever trained under CRF schedule. Only one lever was presented during these training sessions. The presentation of a right or left lever was counterbalanced across rats. A lever press resulted in the illumination of the magazine light which remained on

until the animal entered the magazine, following which a single sucrose pellet was delivered. The CRF schedule lever training continued until rats earned more than 50 pellets in 30 minutes on two consecutive sessions, usually requiring no more than 5 training sessions.

3.2.1.3.2. Symmetrically Reinforced Go/No-go Conditional Visual Discrimination Task

The task was based on Harrison et al.'s (1999) behavioural procedure and is summarised in Fig. 3.1. The task consisted of a total of 80 trials, including 40 Go trials and an equal number of No-go trials presented randomly. Animals were required to discriminate between two visual stimuli of fast (0.1s pulses presented at 5Hz) and slow (0.4s pulses presented at 0.83Hz) synchronised flashings of the stimulus lights located above the levers. For half the animals fast flashing stimuli indicated a Go trial, while the slow flashing stimuli indicated a No-go trial. For the remaining animals fast flashing lights signified a No-go trial and the slow a Go trial, thus allowing the stimulus-reward contingencies to be counterbalanced across the group.

Each trial began with the illumination of the houselight and the initiation of a 5 second inter-trial-interval (ITI) period, following which the discriminative visual stimuli were presented for a 10 second duration. During the first 1.2 seconds of stimulus presentation (the pre-discrimination period) a response on the lever was recorded as an early response. In order to correctly identify the slow stimulus a complete cycle of the stimulus was required to be presented, which is completed only after this time point. Therefore early responses during this time had no further consequence as discrimination between the two stimulus frequencies was unlikely. The presentation of the stimuli continued for a maximum period of 10 seconds until either a response on the lever occurred or animals entered the food magazine. Regardless of trial type, if animals entered the magazine, a 5 second time-out period followed and this response was recorded as an inappropriate magazine entry. During the time-out period the houselight was turned off and any further responses had no consequence on the trial. After the time out period the same trial was then restarted.

If a response occurred on the lever during the stimulus presentation then one of two outcomes followed. If it was currently a Go trial, then a response on the lever resulted in the stimulus light being turned off, and the magazine light being turned on signalling the availability of a food reward. This response was recorded as a correct Go trial. The animal then had a period of 5 seconds to enter the food magazine which would in turn deliver a single pellet and switch the magazine light off. If instead the animal was currently experiencing a No-go trial, a response on the lever resulted in the flashing of stimulus lights being terminated followed by a 5 second time out period of darkness. In this case an incorrect No-go trial was recorded.

If no lever response occurred during the stimulus presentation then again two outcomes

followed depending on whether animals were currently experiencing a Go or No-go trial. If no response occurred during a No-go trial then, at the end of the stimulus presentation the magazine light was switched on and animals had a 5 second period in which to enter the magazine, thus inducing the delivery of a food reward. A correct No-go trial was recorded. If instead animals failed to make a response during a Go trial then a 5 second time-out period of darkness followed and the trial was recorded as an incorrect Go trial.

Response latencies and magazine entrance latencies were also recorded during both Go and No-go trials. Response latencies were measured from the end of the pre-discrimination period of the stimulus presentation until the lever was pressed. Response latencies were recorded as a correct and incorrect response latency during Go and No-go trials respectively. Both correct and incorrect response latencies were utilised to determine the ITI period prior to the following trial. This was achieved by subtracting the response latency from the total duration the stimulus could have been presented (10 seconds) if no lever response had occurred. This duration was then added to the 5 second ITI period. If no response occurred then the ITI duration was simply 5 seconds as the stimulus had been presented for the total duration during the preceding trial. Therefore irrespective of the type of trial that preceded and how the animals responded, the duration between trials always remained constant. This prevented a Go response being favoured due to a possible increase in rate of delivery of reinforcement if a bias occurred.

Magazine latencies were calculated from the time of the correct response; a lever response during Go trials or following the stimulus presentation during No-go trials, until the animal's entry into the food magazine. If animals failed to enter the magazine within 5 seconds, then this was recorded as a magazine omission and animals were not rewarded. All dependent variables recorded in the behavioural procedure are summarised in Table 3.1. Training continued until animals reached a criterion of 85% total correct trials on two consecutive sessions. Animals typically acquired this level of performance following 8 weeks of training. Once this level of accuracy had been achieved, sessions continued for a one week period to ensure accuracy of performance had stabilised prior to testing. During training animals could conduct the behavioural task up to twice per day.

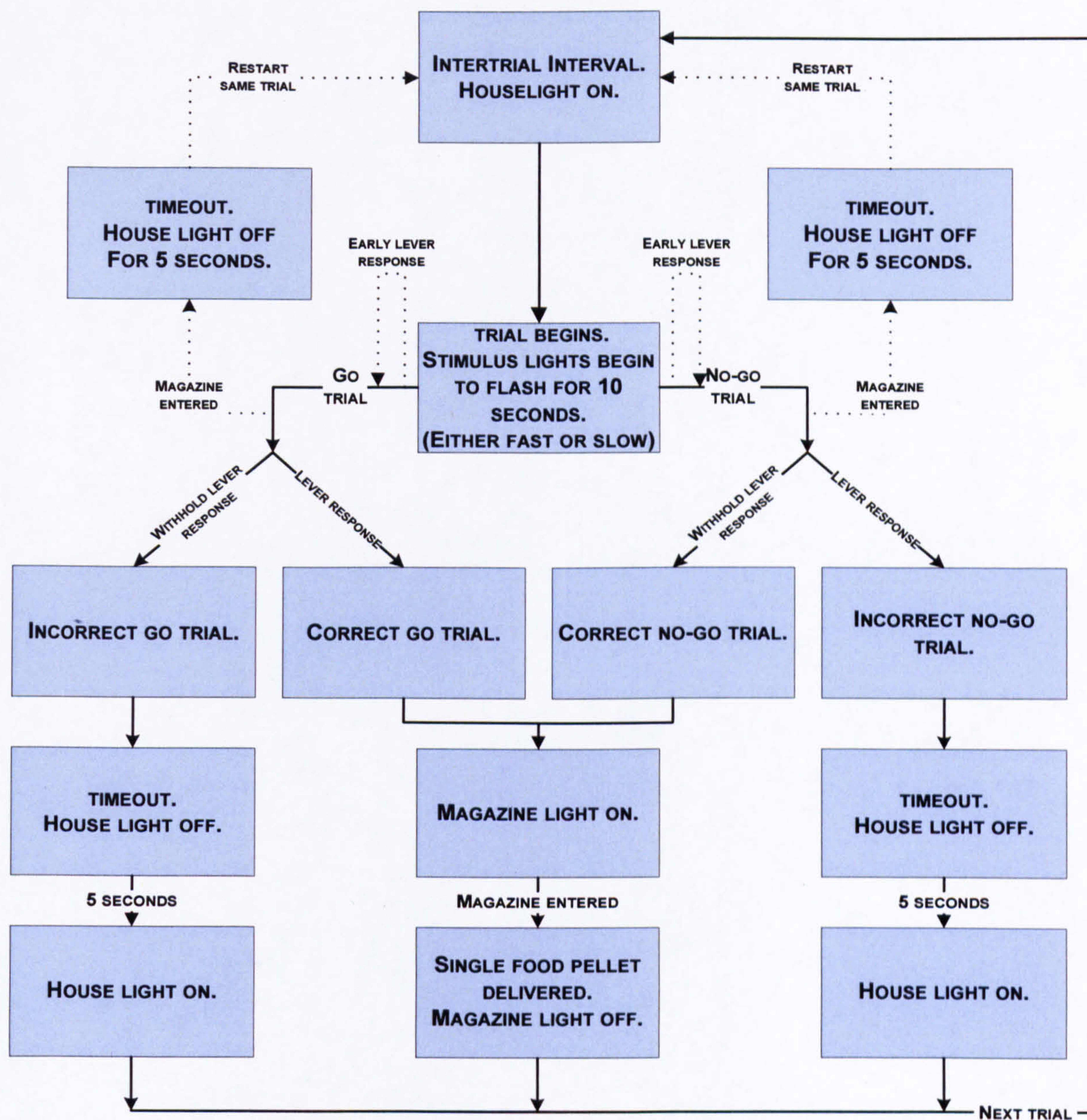


Fig. 3.1: Schematic diagram of the symmetrically reinforced go/no-go conditional visual discrimination task

Table 3.1: Behavioural measures recorded in the symmetrically reinforced go/no-go task

Behavioural Measure	Dependent Variables
Accuracy of Responding	<p>Total percentage correct trials (no. of correct Go trials + correct No-go trials/ 80 * 100)</p> <p>Percentage correct Go trials (no. of correct go trials/ 40 * 100)</p> <p>Percentage correct No-go trials (no. of correct no-go trials/ 40 * 100)</p>
Anticipatory Responding	<p>No. of Go trials with early responses</p> <p>No. of No-go trials with early responses</p> <p>No. of Go trials with inappropriate magazine entries</p> <p>No. of No-go trials with inappropriate magazine entries</p>
Speed of Responding	<p>Correct response latency during Go trials (seconds)</p> <p>Incorrect response latency during No-go trials (seconds)</p> <p>Magazine latency following correct Go trials (seconds)</p> <p>Magazine latency following correct No-go trials (seconds)</p>
Omissions	<p>No. of magazine omissions following correct Go trials</p> <p>No. of magazine omissions following correct No-go trials</p>

3.2.1.4. Design and Procedure

Each experiment followed standard operant testing procedures, outlined in detail previously in the general methodology of Chapter 2, section 2.5.2. Experiments 1 (A-D) employed a within subjects design. For Experiments 1 (B-D) task performance during all manipulations was compared to the stable averaged five day baseline obtained immediately prior to testing. Prior to all subsequent experimental manipulations it was ensured that animals had returned to BL performance before further testing. BL was defined as behaviour in the task deviating no greater

than 5% from the accuracy of performance reached by the subject during the assessment of stability during Experiment 1A. All testing took place during the light phase of their LD cycle between 0800h and 1400h. Experimentation took place over a 12 week period.

3.2.1.4.1. EXPERIMENT 1A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Performance on the task was examined over a three week period, during which animals performed the task once daily. The duration was selected to reflect the duration of future chronic drug studies, where performance would be examined during seven days of baseline, chronic drug treatment and drug withdrawal. Time of operant testing remained constant for each subject during this experimental stage.

3.2.1.4.2. EXPERIMENT 1B: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK WHEN PERFORMANCE IN THE TASK IS ASSESSED TWICE PER TEST DAY

Stability of baseline performance was assessed across a seven day period during which animals performed two operant sessions per test day. Six hours separated daily test sessions during which time animals were returned to their holding room.

3.2.1.4.3. EXPERIMENT 1C: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

To provide comparator profiles for the effects of alterations in primary motivation, Experiment 1C examined the effects of varying levels of deprivation on task performance. The effect of a decrease in motivation for food reward on behavioural parameters in the task was assessed twice using varying procedures. Under the first of these conditions suppression of motivation was achieved through allowing free access to a pre-weighed amount of normal rat chow for one hour immediately prior to testing in their home cage. Under the second condition motivation for sucrose reward delivered during the task was more specifically reduced through allowing animal's free access to a pre-weighed amount sucrose pellets for a 30 minute period prior to testing. A shorter time period of free feeding of sucrose pellets was selected based on preliminary observations indicating a much greater and more rapid consumption of sucrose pellets in comparison to normal rat chow. As with periods of normal feeding, paired housed animals were separated during prefeeding. In order to quantify the level of presatiation, each animal's BW was measured immediately prior and following feeding. The amount of food consumed during the prefeeding periods was also recorded.

An increase in motivation for food reward was assessed during the third experimental condition.

This was achieved through the reduction of food allowance (normally 18.6g) the day prior to testing by 50%. The following test day animals were weighed immediately prior to operant testing to enable assessment of loss of BW. The sequence of manipulations was determined by a Latin square. Between each manipulation, an inter-test interval of at least six days elapsed during which subjects were maintained under their normal baseline operant testing and feeding regime.

3.2.1.4.4. EXPERIMENT 1D: EXAMINATION OF THE EFFECTS OF VARIABLE NO-GO STIMULUS DURATION ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

To determine whether inhibitory responding during No-go trials was dependent on timing behaviour, during a single test session the effects of varying the stimulus duration presented during No-go trials was assessed on performance. During the manipulation the No-go trials stimulus was presented for a duration of 5, 10 or 20 seconds. The duration of stimulus presentation during Go trials remained unchanged from baseline test sessions and was presented for 10 seconds. An equal number of Go trials, and No-go trials of the three varying stimulus durations were randomly presented during 80 trials. Therefore each trial type was presented on twenty occasions during the test session.

3.2.1.5. Statistical Analysis

For all statistical procedures data were assessed for normality and transformed prior to analysis where necessary (see also section 2.7.). Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction.

3.2.1.5.1. Training

In order to compare the independent acquisition of Go and No-go trials the number of sessions required to reach above chance level of accuracy on each trial (65% total correct trials on two consecutive sessions) (Siegel, 1956) were analysed by a repeated measures t-test.

3.2.1.5.2. EXPERIMENT 1A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Examination of the stability of performance across all behavioural parameters (accuracy of performance, anticipatory responding, speed of responding and omissions) was conducted by a one-way repeated measures ANOVA, with test day as the within subject factor.

3.2.1.5.3. EXPERIMENT 1B: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK WHEN PERFORMANCE IN THE TASK IS ASSESSED TWICE PER TEST DAY

Behavioural measures across the seven day period during time one and time two performances, were independently compared to the prior five day average baseline performance. This was achieved by conducting a repeated measures one-way ANOVA on time one and time two behavioural measures, with test session as the within subject factor with 8 levels (average BL, plus seven test days).

3.2.1.5.4. EXPERIMENT 1C: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

For each experimental condition the five day average baseline performance prior to the manipulation was compared directly to the effects of alterations in primary motivation on behaviour. Comparisons across all parameters were conducted using repeated measures t-tests. Comparison of differences in food intake and weight gained across manipulations were also conducted by a repeated measures t-test. Weight was assessed as both an absolute weight gain(g) (or loss) and percentage change from the weight recorded either immediately prior to the prefeeding period or the day prior to the feeding manipulation (in the case of assessment of acute-induced increase in motivation).

3.2.1.5.5. EXPERIMENT 1D: EXAMINATION OF THE EFFECTS OF VARIABLE NO-GO STIMULUS DURATION ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Variation in stimulus duration during No-go trials during a single test session was compared to average prior BL performance. Overall accuracy, anticipatory responding and speed of responding during Go trials were compared to BL performance using repeated measures t-tests. Assessments of accuracy, anticipatory responding, speed of responding and omissions during No-go trials relative to BL performance were conducted instead by a series of one-way repeated measures ANOVAs, with stimulus duration as the within subject factor with 4 levels (No-go (BL 10secs duration), No-go (5secs duration), No-go (10secs duration), No-go(20secs duration)). Due to the reduction in the number of Go and No-go trials during the manipulation, the frequency of trials with anticipatory responding and omissions was expressed as a proportion of trial number prior to analysis to enable valid comparison to BL.

For Experiments 1 (A, B, D) all significant main effects were followed where appropriate by Bonferroni post hoc comparisons in order to identify the location of significant difference. If data could not be successfully transformed, then the non-parametric equivalent Friedman and Wilcoxon Signed Ranks tests were employed. In all cases of statistical analysis, α was set at $p < 0.05$.

3.3. RESULTS

Magazine omissions (the failure to collect reward) following a correct response during Go trials rarely occurred throughout experimentation and therefore analysis of this behaviour was not conducted.

3.3.1. Training

Animals required 47.83 ± 3.74 (mean \pm SEM) sessions to reach criterion performance of 85%

total percentage correct trials. Comparison of acquisition of Go and No-go trials indicated that animals reached above chance level of performance during Go trials following significantly fewer training sessions in comparison to the sessions required to reach this level of accuracy during No-go trials ($t = -7.471$, $df = 6$, $p < 0.001$) (No. of training sessions to reach 65% correct Go Trials: 2.43 ± 0.20 ; No. of training sessions to reach 65% correct No-go Trials: 40.57 ± 5.25).

3.3.2. EXPERIMENT 1A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

3.3.2.1. Accuracy of Responding

Overall accuracy of performance in the go/no-go task remained stable across the three week period varying between 84-90% total correct trials ($F(3.542, 17.712) = 0.827$, N.S.) (see Fig. 3.2a). Stable furthermore, was the independent performance on Go and No-go trials, as supported by the absence of significant main effects of test day for both Go trials ($F(2.845, 14.225) = 1.063$, N.S.) and No-go trials ($F(3.191, 15.953) = 0.642$, N.S.). As illustrated in Figs. 3.2 (b-c), accuracy on Go trials was consistently greater than performance during No-go trials, with almost 100% accuracy of responding during these trials displayed. In contrast, performance during No-go trials ranged between 65-80% with considerably greater variability across animals in accuracy of responding on these trials.

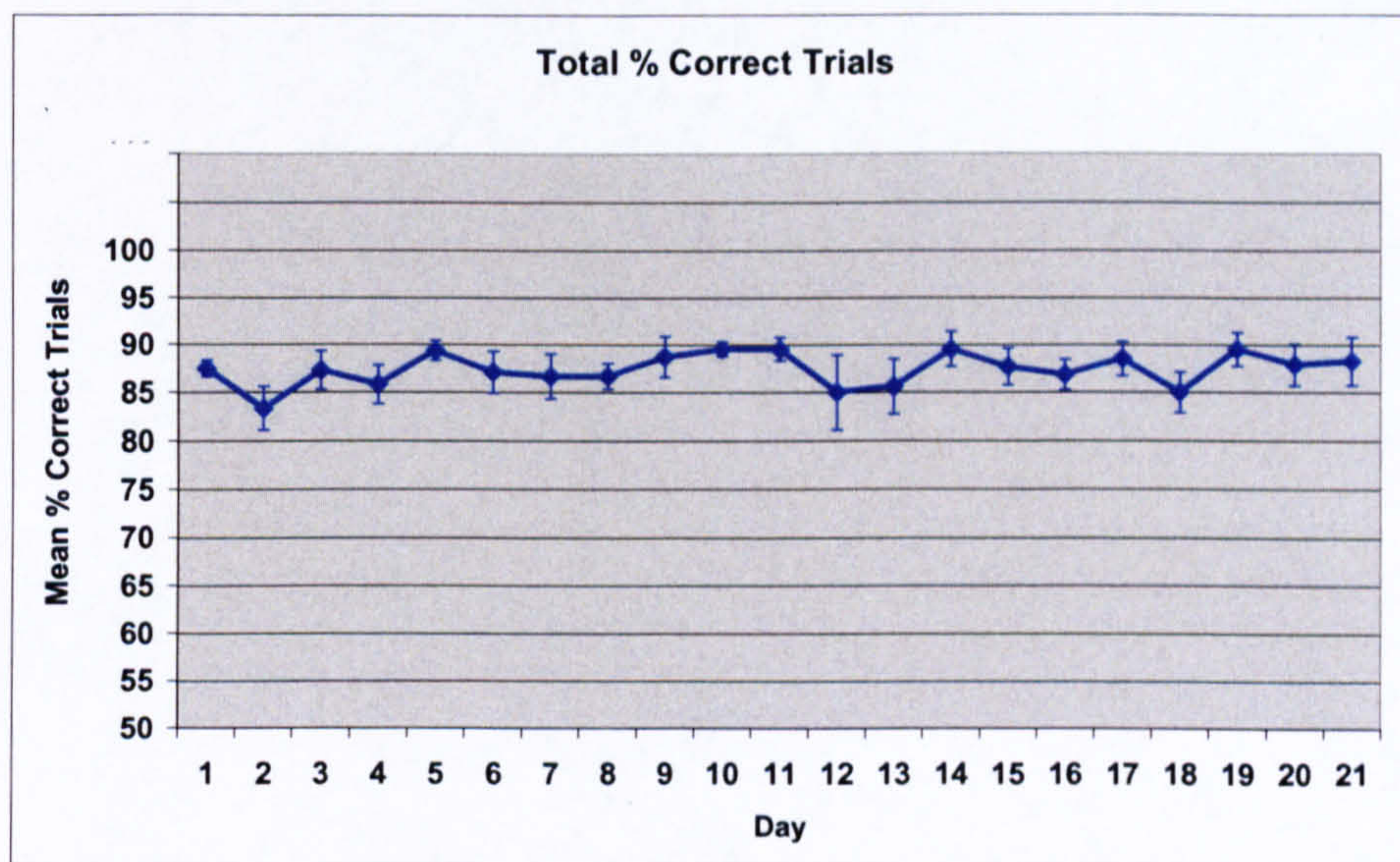


Fig. 3.2 (a)

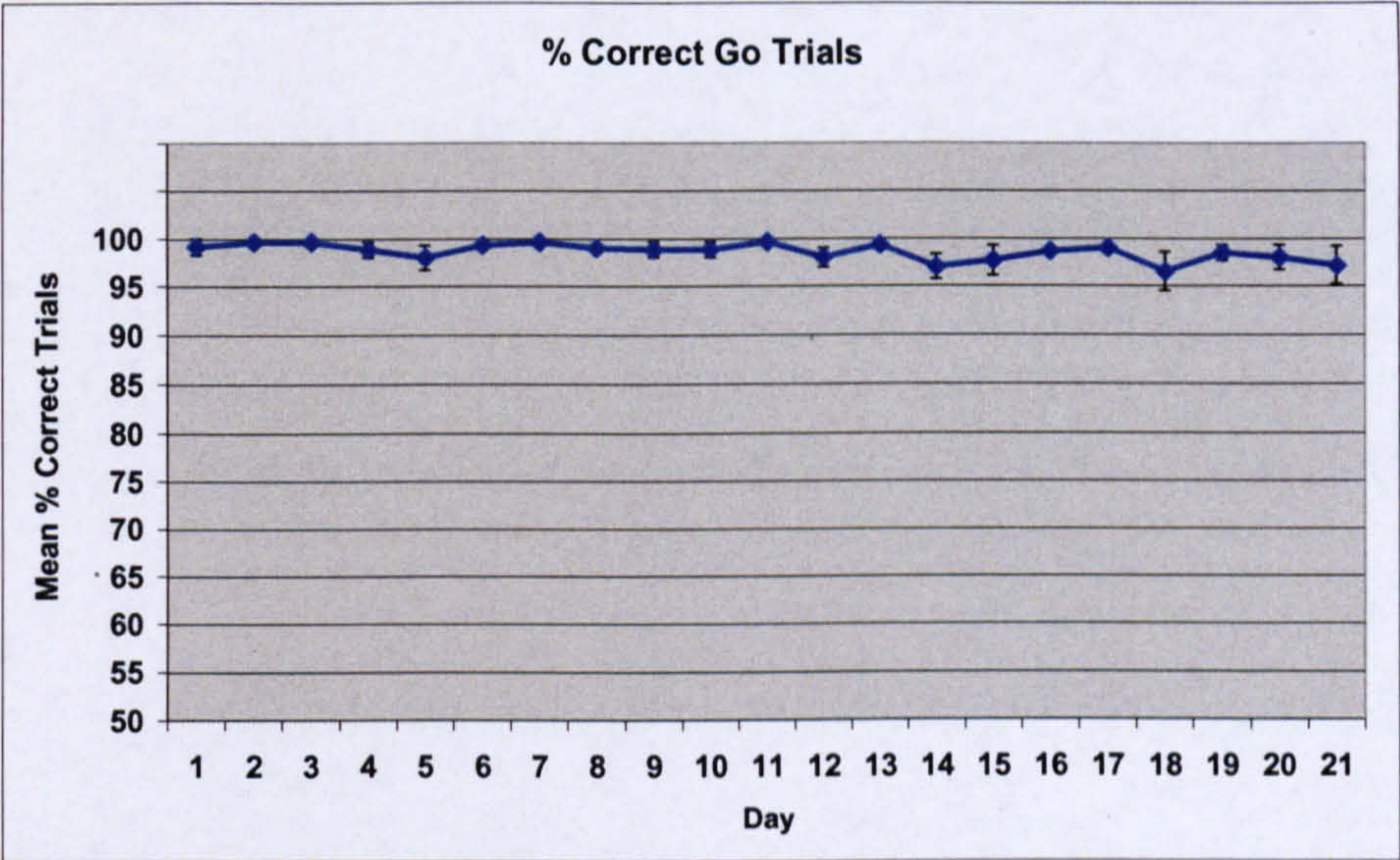


Fig. 3.2 (b)

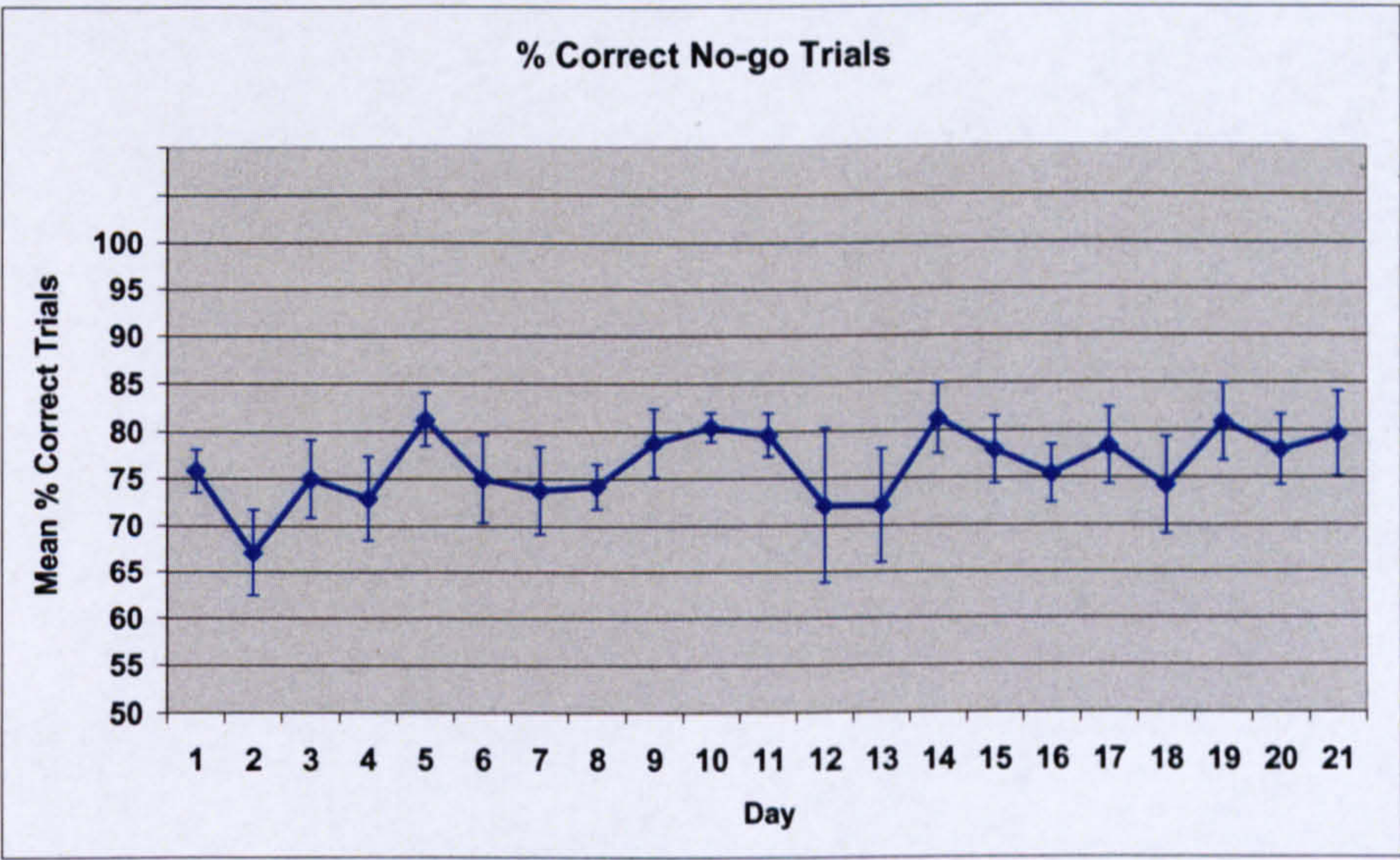


Fig. 3.2 (c)

Fig. 3.2 (a-c): Accuracy of responding: Stability of percentage correct Total trials (a), Go trials (b) and No-go trials (c) across the 21 day period. Each point represents the mean percentage \pm SEM.

3.3.2.2. Anticipatory Responding

Analysis revealed no significant main effect of test day for frequency of early responding during Go ($F(3.510, 17.550) = 0.804$, N.S.) and No-go trials ($F(2.518, 12.591) = 0.8434$, N.S.), indicating stability of performance across the three week period (see Figs. 3.3 (a-b)). Comparably, non-significant main effects of test day were indicated for inappropriate magazine entries during both Go ($F(1.720, 8.599) = 0.843$, N.S.) and No-go trials ($F(2.518, 12.591) = 1.130$, N.S.) (Figs. 3.3 (c-d)). As clearly shown in Figs. 3.3 (a-d), the frequency of early responding was greatest during Go trials whilst inappropriate magazine entries were more often observed during No-go trials.

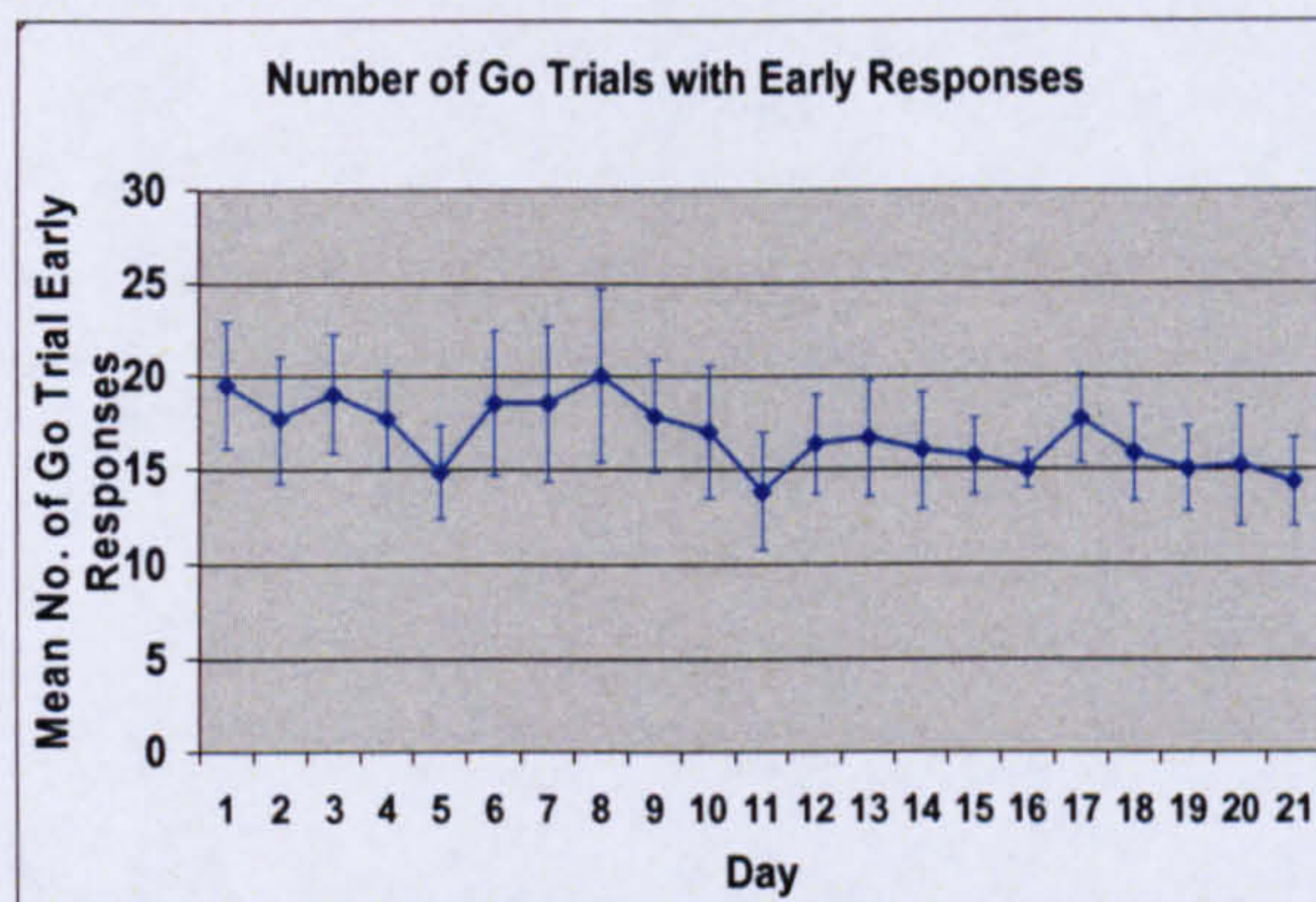


Fig. 3.3 (a)

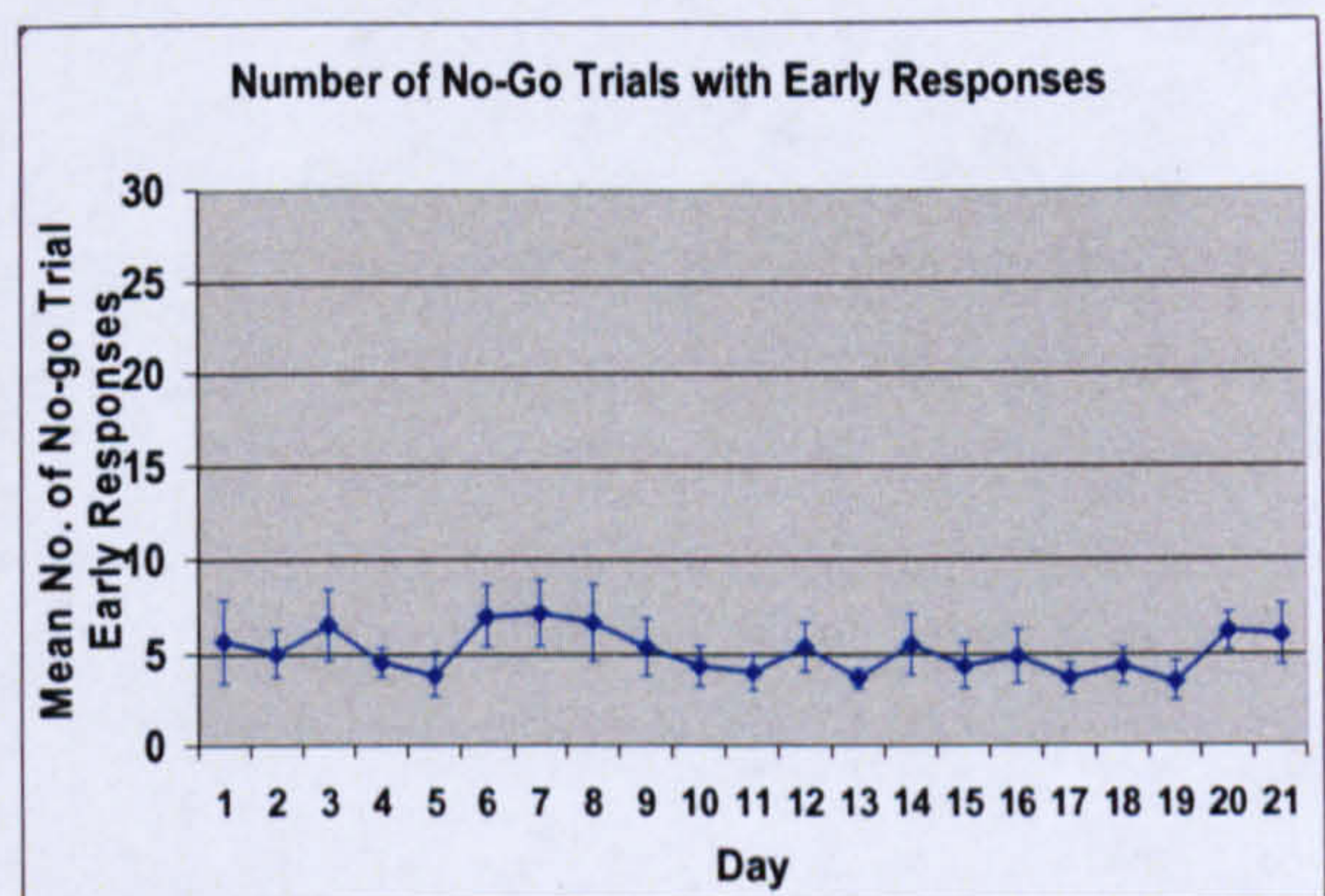


Fig. 3.3 (b)

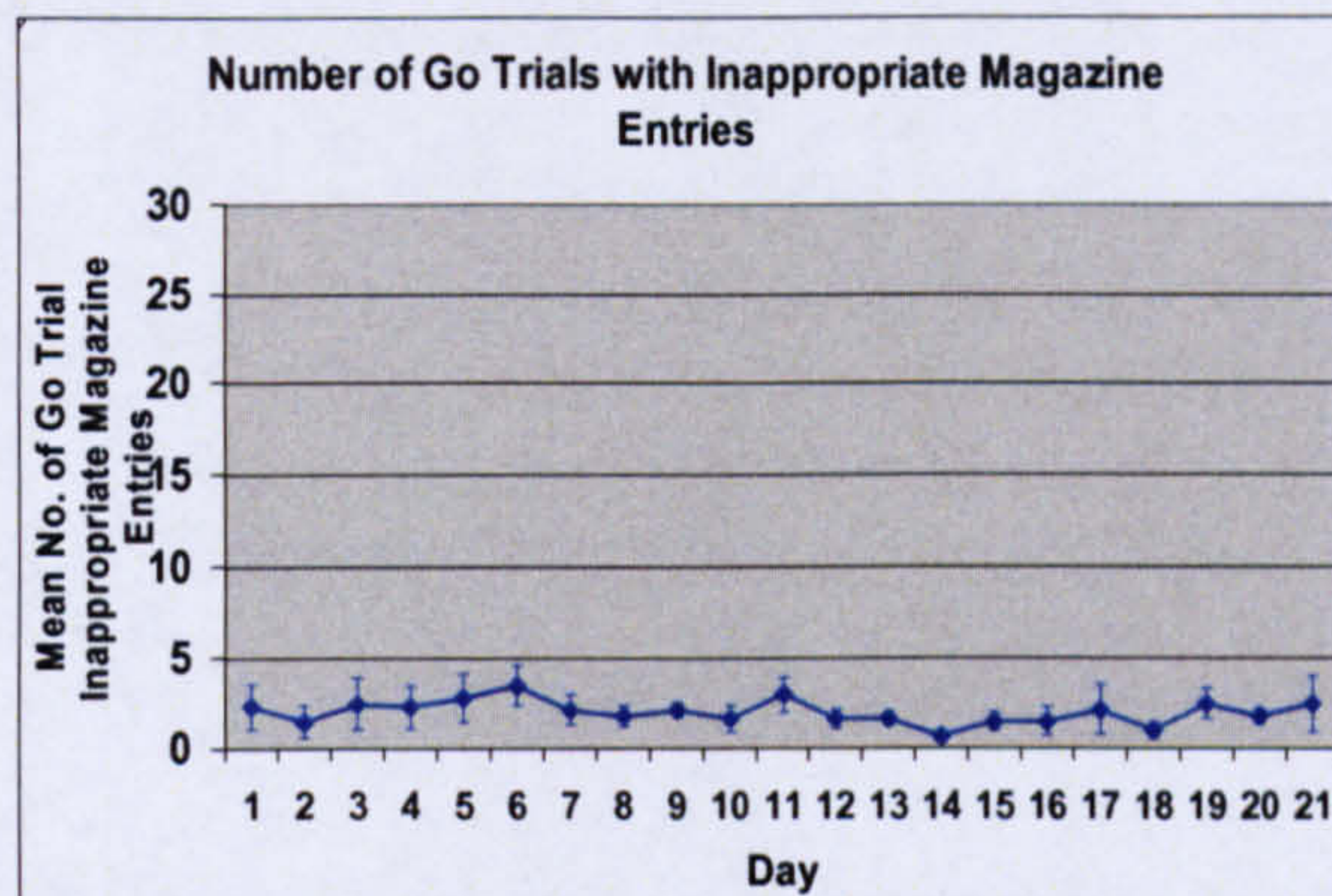


Fig. 3.3 (c)

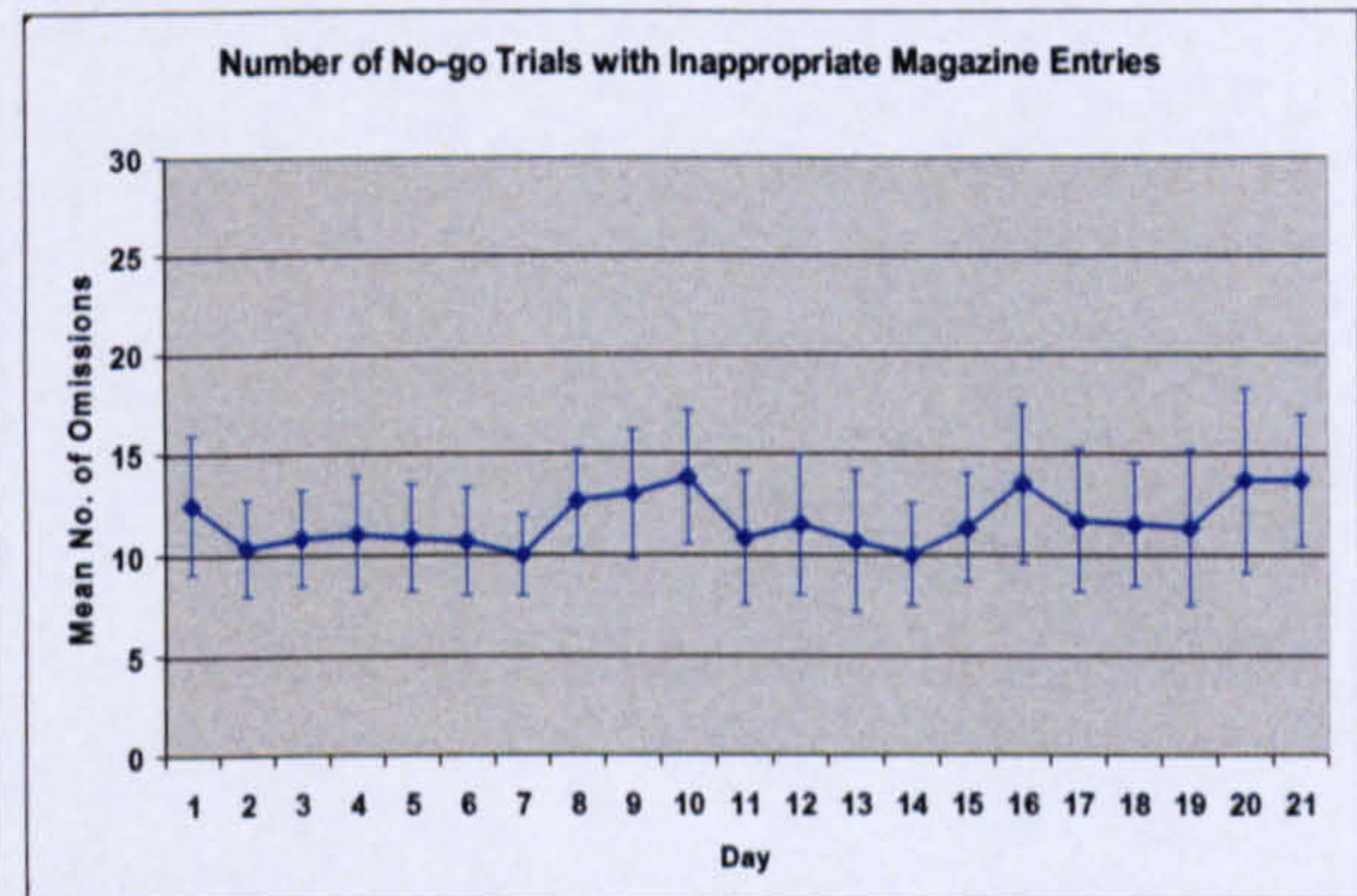


Fig. 3.3 (d)

Fig. 3.3 (a-d): Anticipatory responding: stability of early responses during Go trials (a) and No-go trials (b) across the 21 day period. Anticipatory Responding: stability of inappropriate magazine entries during Go trials (c) and No-go trials (d) across the 21 day period. Each point represents the mean number of trials \pm SEM.

3.3.2.3. Speed of Responding

Figs. 3.4 (a-d) illustrate the stability of speed of responding measures within the go/no-go task during the three week period. No main effect of test day was observed for latency to correctly respond during Go trials ($F(3.304, 16.520) = 0.884$, N.S.) and incorrectly respond during No-go trials ($F(3.830, 19.150) = 1.211$, N.S.).

Analysis of the speed at which animals collected reward furthermore indicated stability of performance. No significant main effect of test day was revealed for latency to collect reward following both correct Go and No-go trials ($F(3.522, 17.610) = 1.354$, N.S.; $F(3.493, 17.464) = 1.339$, N.S., respectively).

3.3.2.4. Omissions

Non-parametric analysis of the failure to collect reward during No-go trials indicated stability of this behavioural parameter across the assessed time period ($X^2 = 27.400$, $df = 20$, N.S.) (see Fig 3.5).

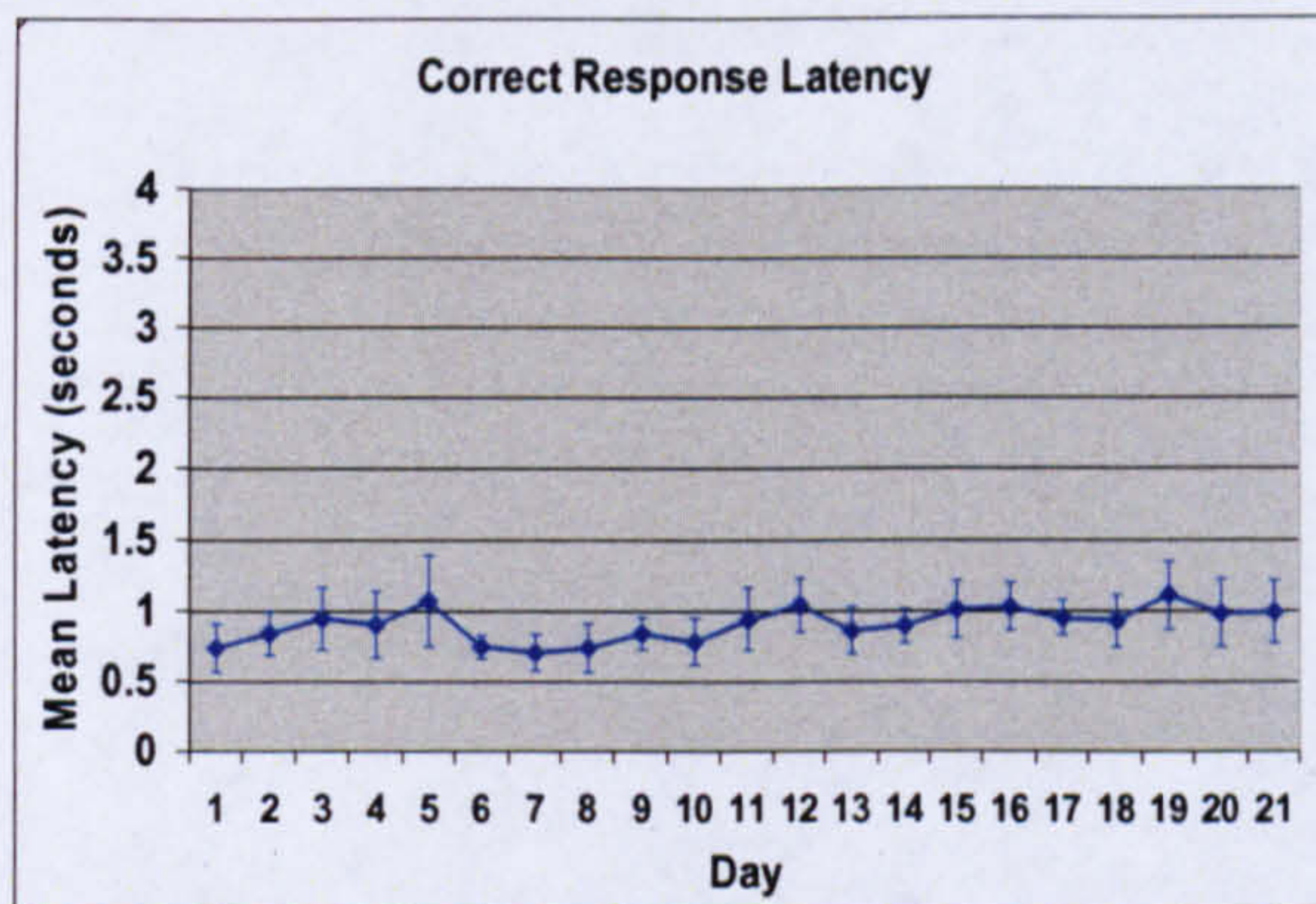
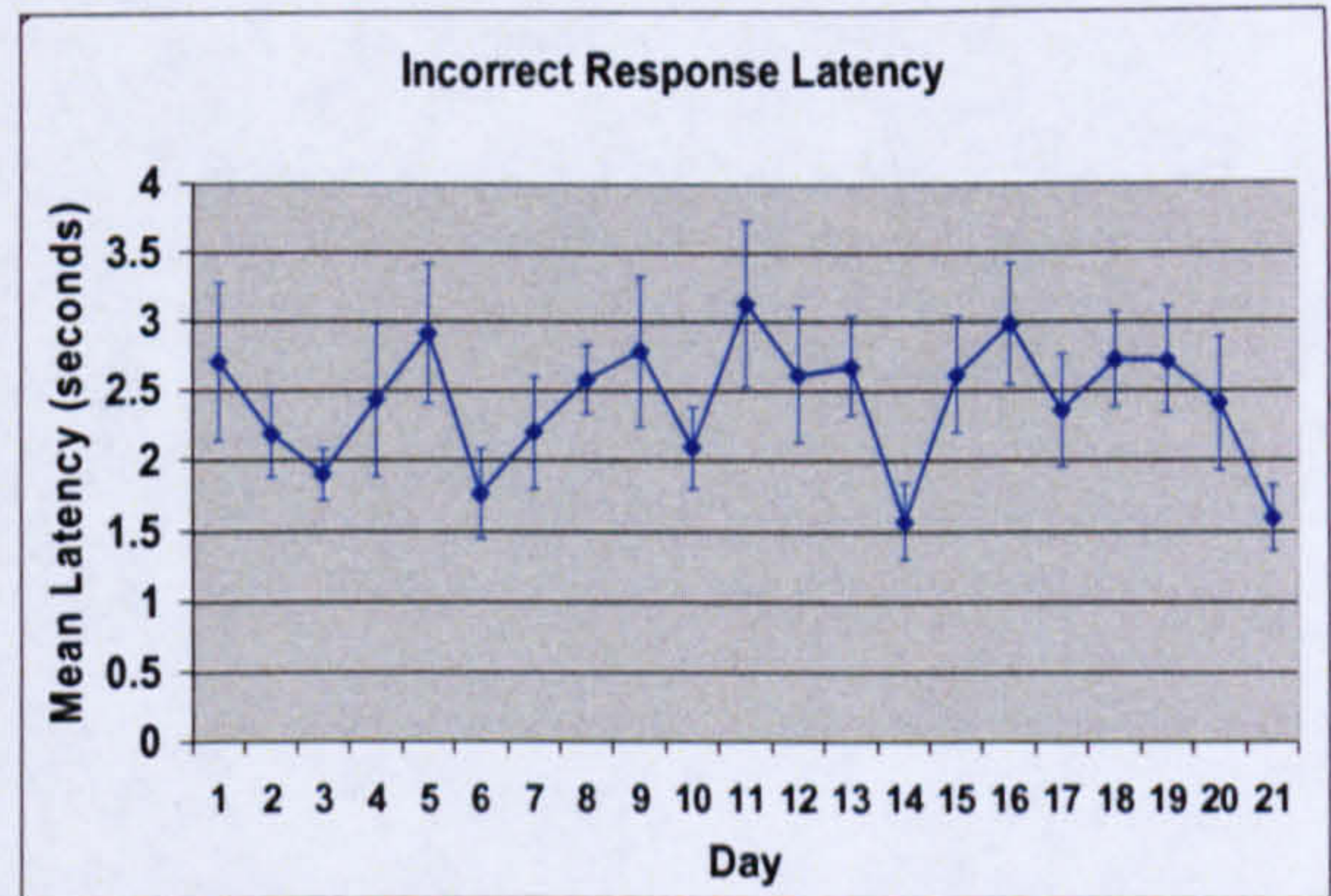
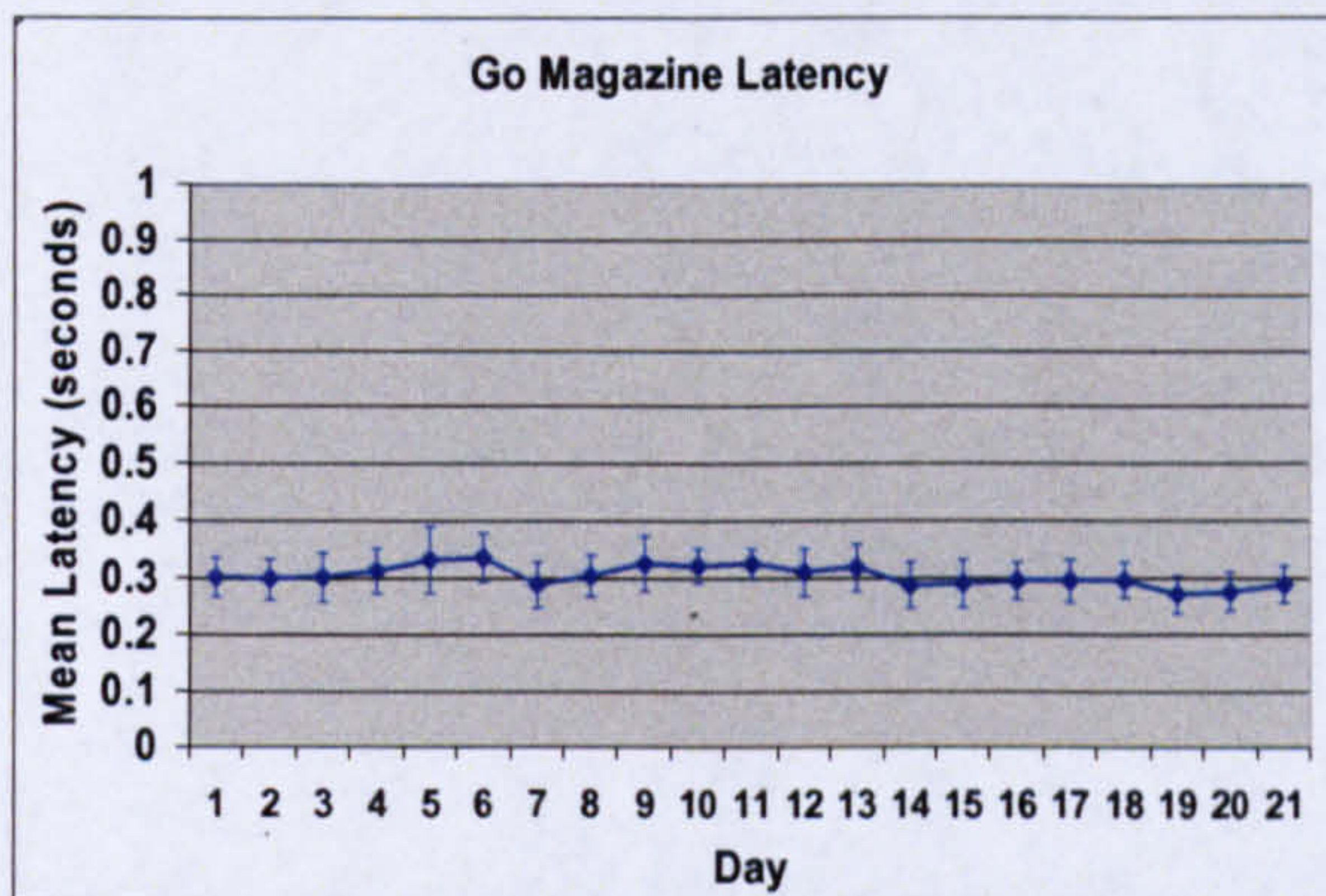
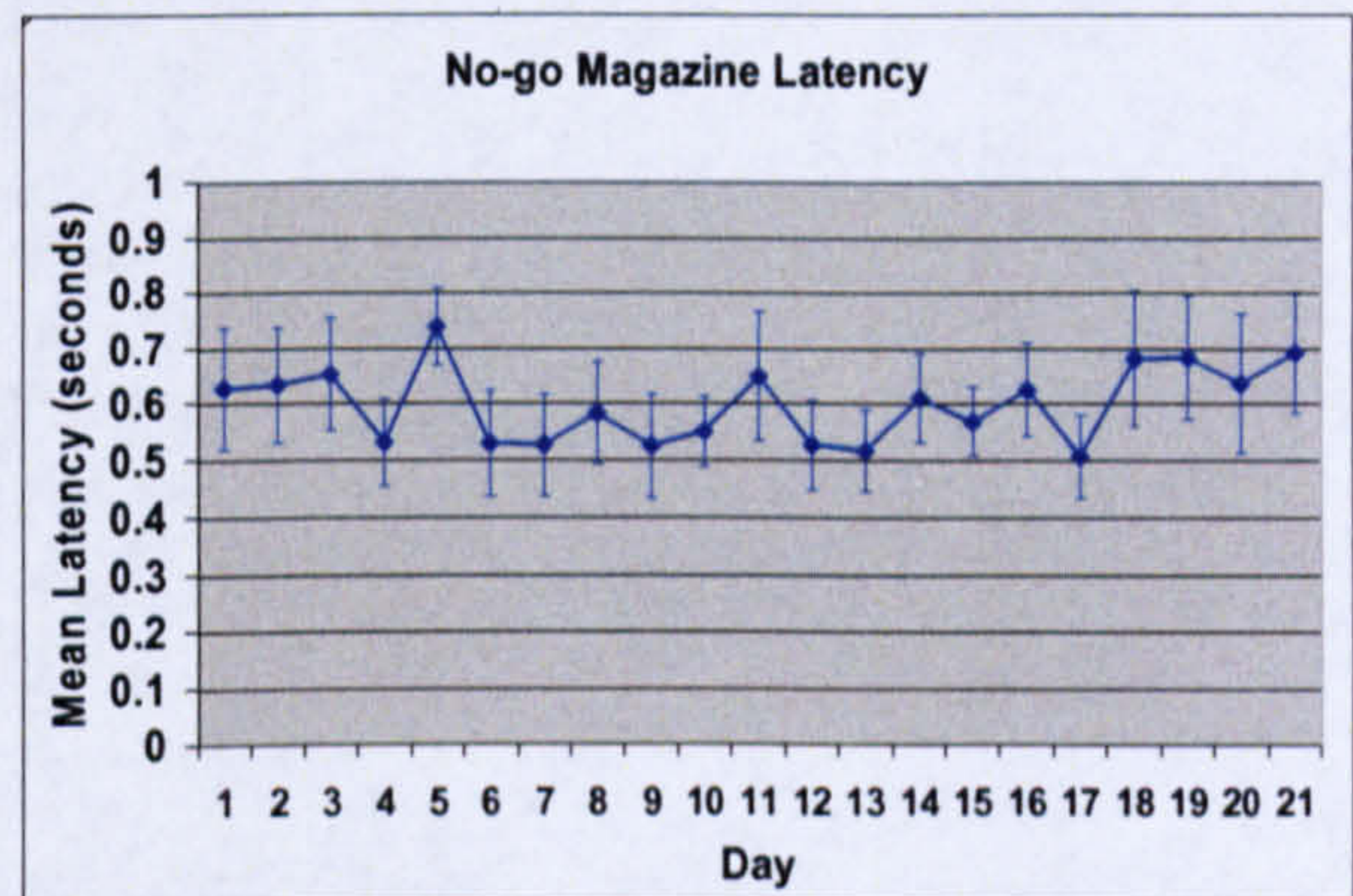
**Fig. 3.4 (a)****Fig. 3.4 (b)****Fig. 3.4 (c)****Fig. 3.4 (d)**

Fig. 3.4 (a-d): Speed of responding: the stability of latency in seconds to respond correctly during Go trials (a) and incorrectly during No-go trials (b) across the 21 day period. Speed of Responding: the stability of latency in seconds to collect reward following correct Go trials (c) and No-go Trials (d) across the 21 day period. Each bar represents the mean latency in seconds \pm SEM.

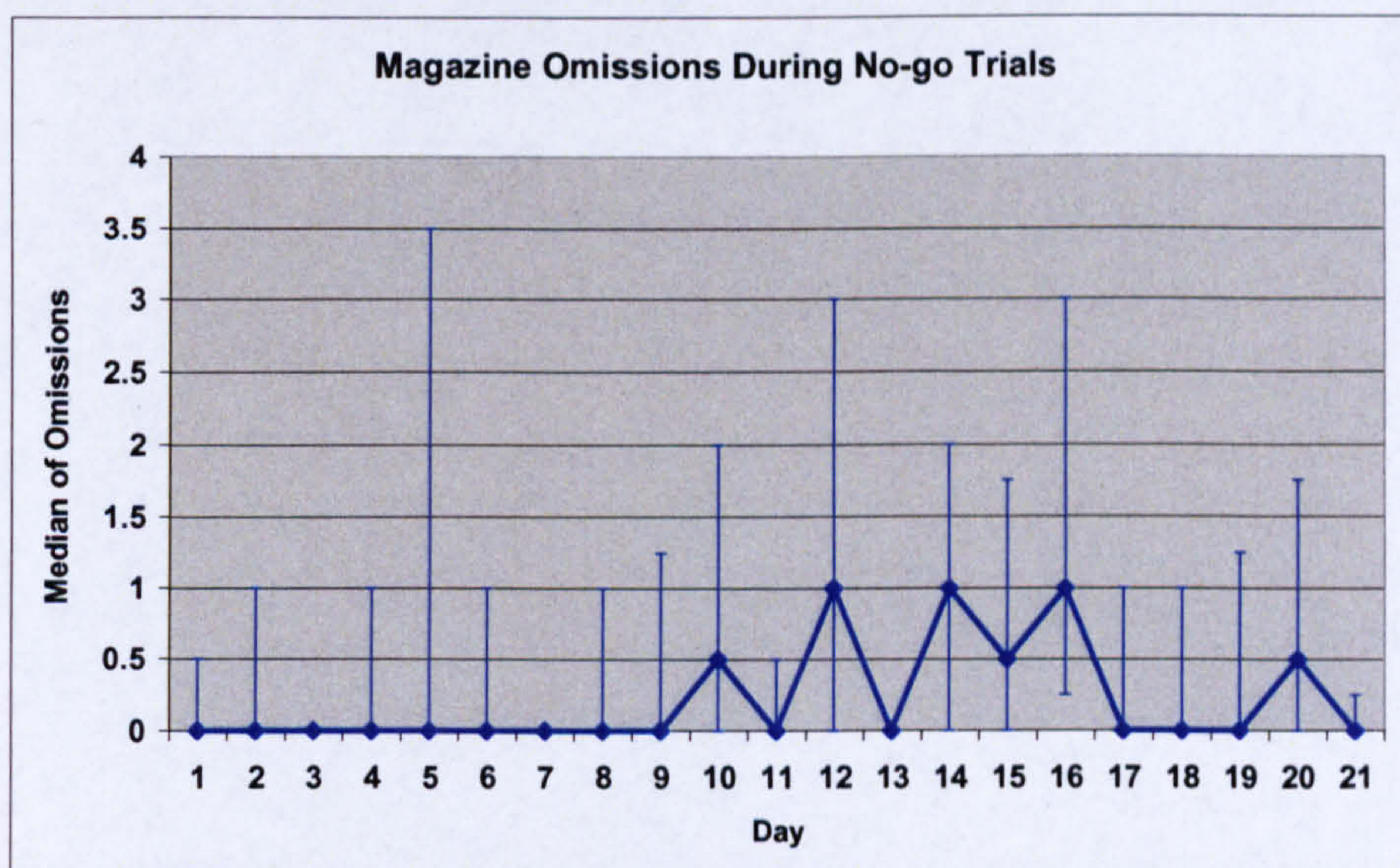


Fig. 3.5: Omissions: the stability of frequency of magazine omission during no-go trials across the 21 day period. Each point represents the median frequency \pm Inter Quartile Range.

3.3.3. EXPERIMENT 1B: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK WHEN PERFORMANCE IN THE TASK IS ASSESSED TWICE PER TEST DAY

3.3.3.1. Accuracy of Responding

Figs. 3.6 (a-c) illustrate the stability of accuracy of performance in the go/no-go task when assessed twice per test day across a seven day period. Analysis of total percentage correct trials demonstrated no significant changes in overall accuracy in the task in comparison to baseline during time one ($F(7,35) = 1.518$, N.S.) and time two ($F(7,35) = 1.602$, N.S.) performance. Independent performance during both Go and No-go trials furthermore remained stable when animals performed the task twice per test day. No significant main effect of test day was indicated for Go trials (time one: $F(7,35) = 0.715$, N.S.; time two: $F(7,35) = 1.520$, N.S.) and No-go trials (time one: $F(7,35) = 1.225$, N.S.; time two: $F(7,35) = 0.961$, N.S.) during both time periods.

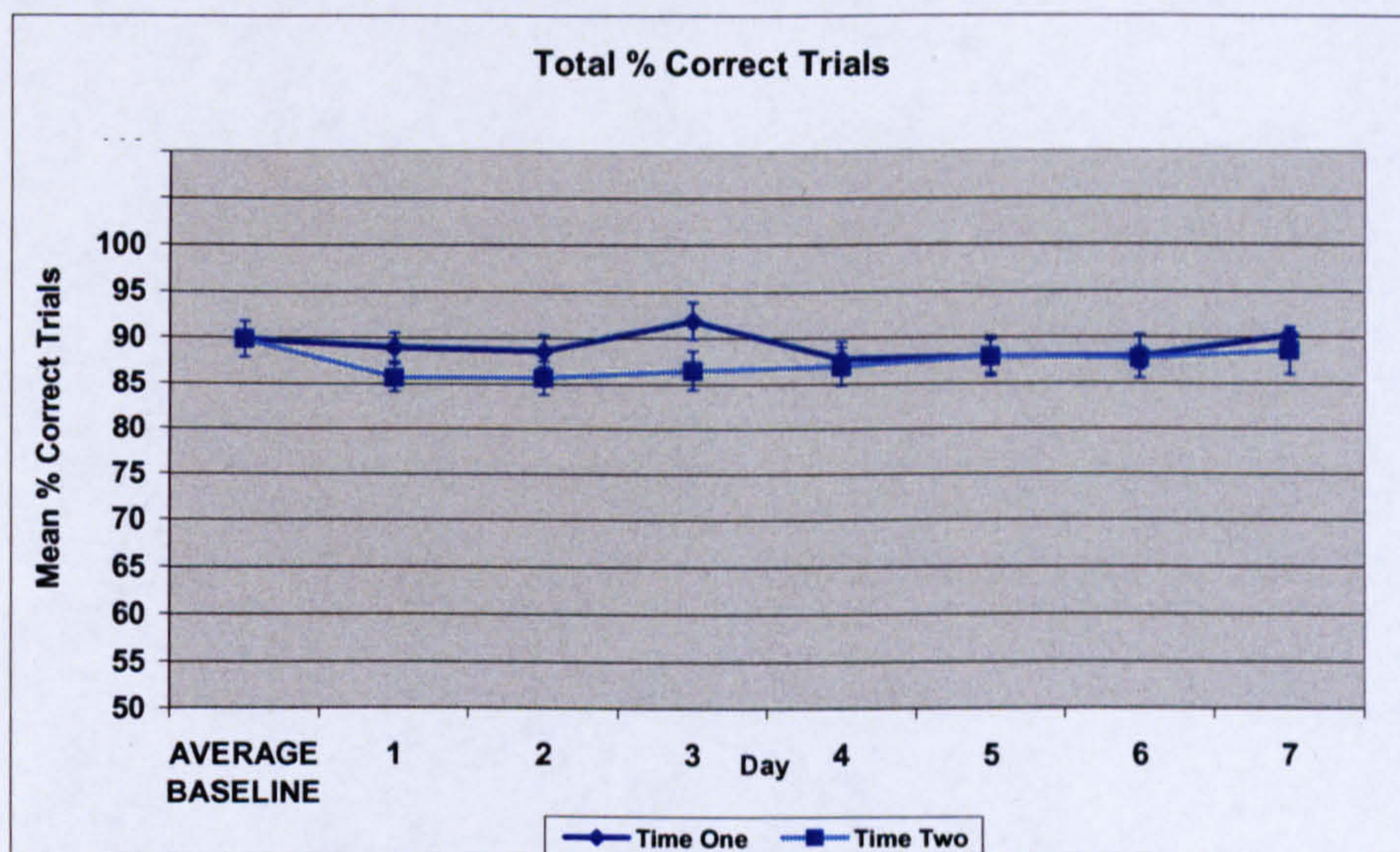


Fig. 3.6 (a)

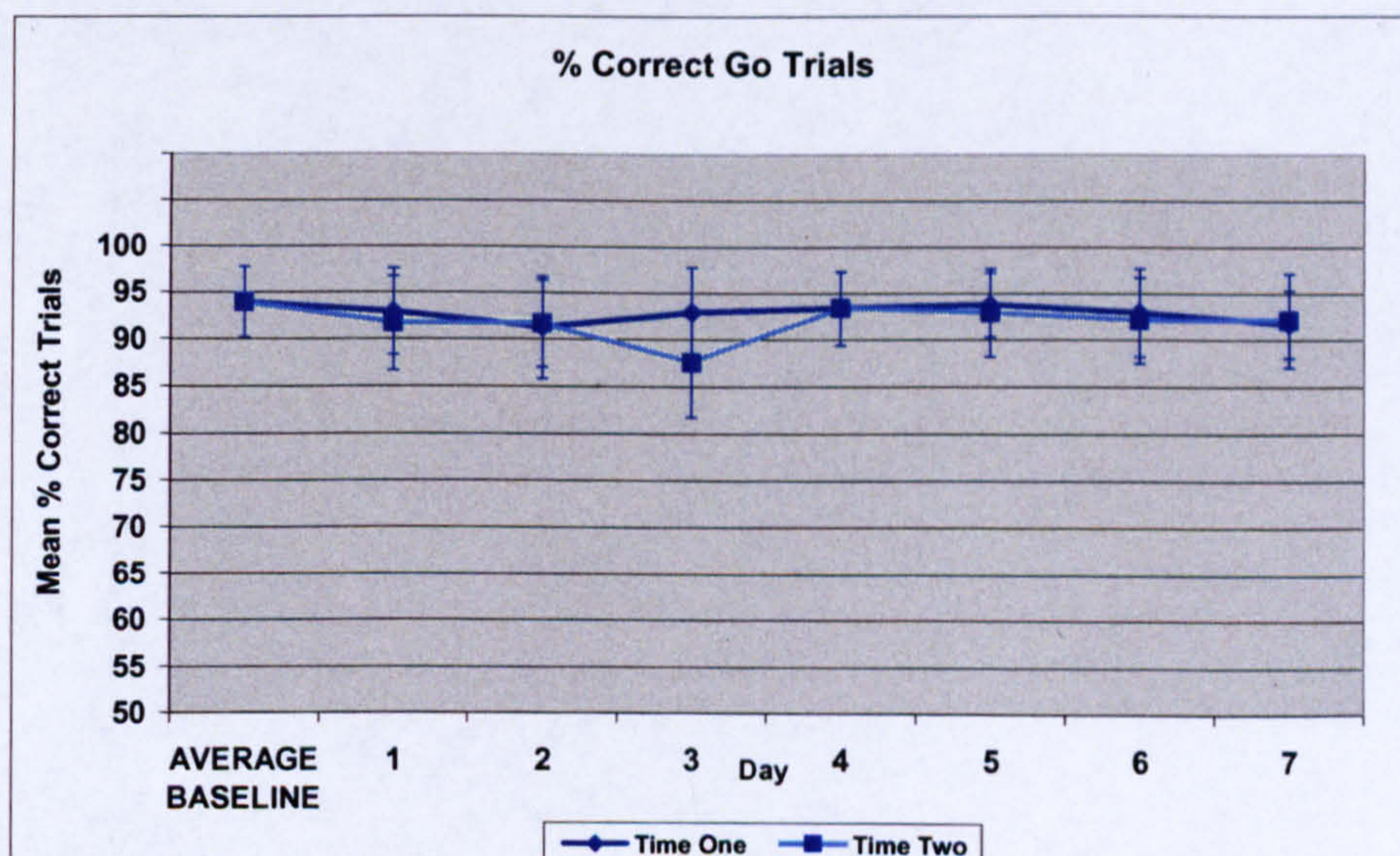


Fig. 3.6 (b)

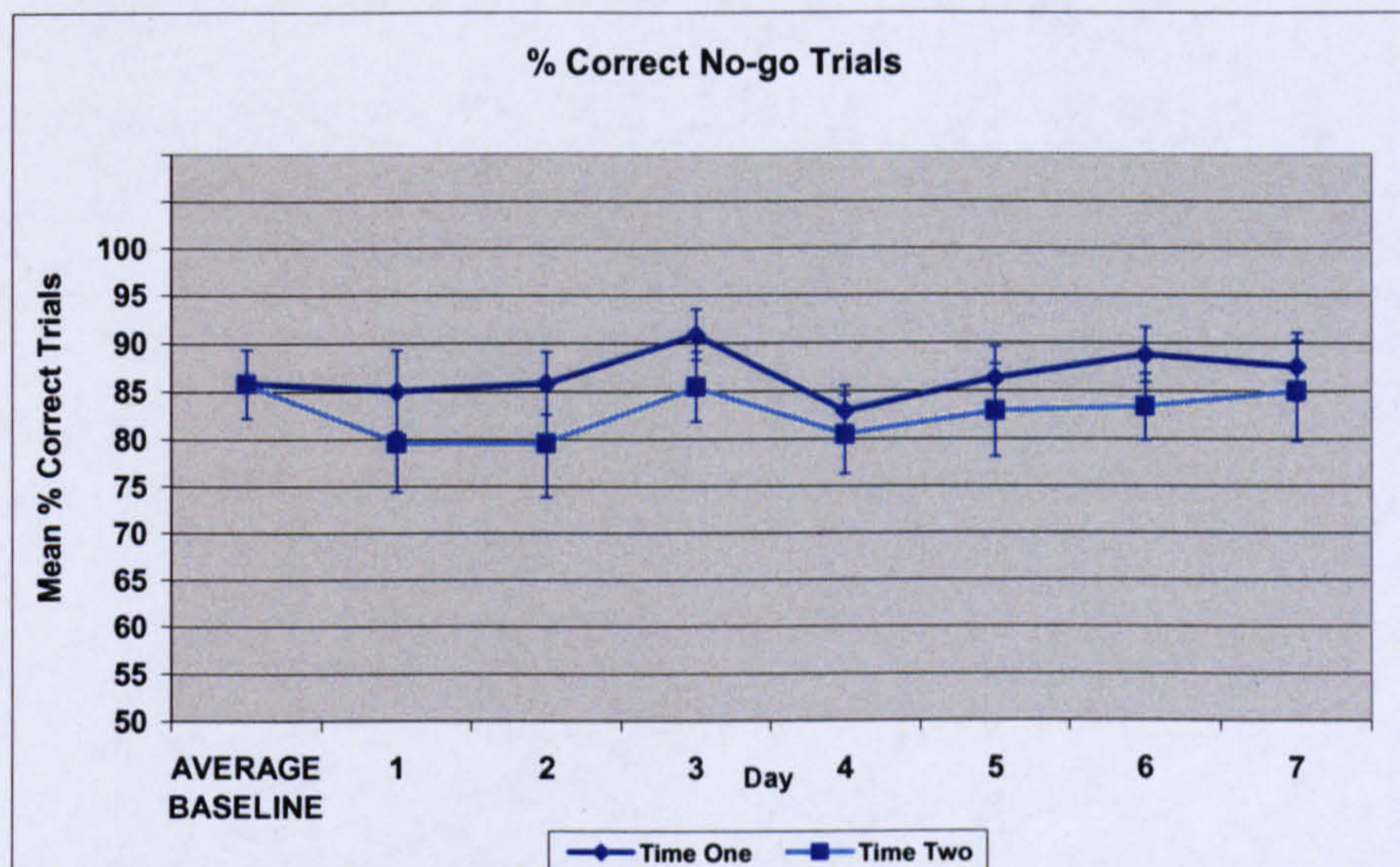


Fig. 3.6 (c)

Fig. 3.6 (a-c): Accuracy of Responding: stability of percentage correct Total trials (a), Go trials (b) and No-go trials (c) across a seven day period during which animals performed the task twice per test day. Each point represents the mean percentage \pm SEM.

3.3.3.2. Anticipatory Responding

Analysis of early responding during time one and time two revealed no significant main effect of test day for this anticipatory measure during both Go (time one: $F(7,35) = 0.911$, N.S.; time two: $F(7,35) = 1.152$, N.S.) and No-go trials (time one: $F(7,35) = 0.1054$, N.S.; time two: $F(7,35) = 1.404$, N.S.). Performing the task twice per test day furthermore had no significant effect on the frequency of inappropriate magazine entries during Go (time one: $F(7,35) = 0.689$, N.S.; time two: $F(7,35) = 1.133$, N.S.) and No-go trials (time one: $F(7,35) = 1.061$, N.S.; time two: $F(7,35) = 1.176$, N.S.). Table 3.2 summarises the levels of anticipatory responding in the task displayed by animals during time one and time two in comparison to baseline performance.

3.3.3.3. Speed of Responding

Latency to respond correctly during Go trials and incorrectly during No-go trials did not differ significantly from baseline performance during time one and time two across the seven day period. No significant main effect of test day was indicated for correct (time one: $F(7,35) = 0.767$, N.S.; time two: $F(7,35) = 0.338$, N.S.) and incorrect response latency (time one: $F(7,35) = 1.653$, N.S.; time two: $F(7,35) = 1.096$, N.S.).

Analysis further revealed no significant main effect of test day for latency to collect reward during Go (time one: $F(7,35) = 1.088$, N.S.; time two: $F(7,35) = 1.328$, N.S.), and No-go trials (time one: $F(7,35) = 0.485$, N.S.; time two: $F(7,35) = 0.789$, N.S.) during task performance at both time periods. Table 3.3 summarises speed of responding measures in the task during time one and time two.

3.3.3.4. Omissions

No significant main effect of test day was observed on failure to collect reward during no-go trials during performance in that task at time one ($X^2 = 4.555$, $df = 7$, N.S.) and time two ($X^2 = 4.877$, $df = 7$, N.S.) (see Table 3.3).

Table 3.2: Stability of anticipatory responding in the symmetrically reinforced go/no-go task when performing the task twice per day

Behavioural Measure		Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go Trials With Early Responses	Time 1	14.07 ± 3.27	12.83 ± 3.39	14.5 ± 3.06	11.50 ± 2.77	11.67 ± 3.08	12.33 ± 3.68	13.50 ± 3.07	12.00 ± 3.15
	Time 2		12.50 ± 3.91	11.17 ± 2.53	11.33 ± 4.11	11.83 ± 2.71	10.17 ± 3.40	10.83 ± 2.99	13.00 ± 3.02
No-go Trials With Early Responses	Time 1	3.80 ± 1.09	4.00 ± 1.63	4.83 ± 1.72	3.67 ± 1.90	4.50 ± 1.23	3.17 ± 1.05	2.50 ± 1.23	3.83 ± 0.91
	Time 2		5.00 ± 1.86	3.67 ± 1.33	3.33 ± 1.58	5.33 ± 1.87	4.50 ± 1.59	2.67 ± 1.12	4.67 ± 1.62
Go Trials With Magazine Entries	Time 1	1.27 ± 0.47	1.33 ± 0.42	2.00 ± 0.52	1.33 ± 0.81	1.17 ± 0.40	2.17 ± 1.14	1.83 ± 0.65	1.17 ± 0.40
	Time 2		2.17 ± 0.91	1.33 ± 0.88	2.33 ± 0.71	2.00 ± 0.63	2.50 ± 1.36	1.50 ± 0.50	0.33 ± 0.21
No-go Trials With Magazine Entries	Time 1	8.53 ± 3.02	6.83 ± 3.36	8.50 ± 3.40	9.00 ± 4.00	8.83 ± 3.56	9.67 ± 4.01	9.17 ± 4.42	8.17 ± 3.43
	Time 2		7.67 ± 3.49	7.67 ± 2.99	8.67 ± 3.61	8.33 ± 2.65	10.83 ± 3.47	10.17 ± 3.70	9.00 ± 3.54

Table 3.2: Each value represents the mean frequency ± SEM.

Table 3.3: Stability of speed of responding and omissions in the symmetrically reinforced go/no-go task when performing the task twice per day

Behavioural Measure		Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Correct Response Latency	Time 1	1.11 ± 0.30	1.10 ± 0.26	1.13 ± 0.37	1.24 ± 0.33	1.22 ± 0.28	1.03 ± 0.32	1.18 ± 0.43	0.95 ± 0.22
	Time 2		1.33 ± 0.34	1.10 ± 0.30	1.34 ± 0.41	1.11 ± 0.24	1.27 ± 0.32	1.23 ± 0.40	1.22 ± 0.33
Incorrect Response Latency	Time 1	1.46 ± 0.29	2.91 ± 0.70	1.90 ± 0.40	2.50 ± 0.65	1.15 ± 0.22	2.05 ± 0.41	1.41 ± 0.33	1.99 ± 0.23
	Time 2		1.75 ± 0.29	2.21 ± 0.40	2.61 ± 0.46	1.86 ± 0.59	1.36 ± 0.42	1.96 ± 0.46	1.92 ± 0.51
Go Magazine Latency	Time 1	0.27 ± 0.04	0.26 ± 0.04	0.26 ± 0.04	0.29 ± 0.04	0.25 ± 0.04	0.27 ± 0.04	0.29 ± 0.05	0.26 ± 0.03
	Time 2		0.26 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.26 ± 0.03	0.27 ± 0.04	0.24 ± 0.04	0.24 ± 0.03
No-go Magazine Latency	Time 1	0.69 ± 0.13	0.66 ± 0.09	0.59 ± 0.09	0.66 ± 0.08	0.60 ± 0.08	0.59 ± 0.08	0.65 ± 0.16	0.72 ± 0.22
	Time 2		0.61 ± 0.08	0.59 ± 0.08	0.78 ± 0.14	0.64 ± 0.08	0.68 ± 0.10	0.76 ± 0.14	0.66 ± 0.15
No-go Trial Magazine Omission	Time 1	1.03 ± 0.66	3.50 ± 1.44	2.50 ± 0.29	1.50 ± 0.29	2.33 ± 0.94	1.33 ± 0.24	1.50 ± 0.40	2.33 ± 0.94
	Time 2		3.67 ± 0.58	2.00 ± 1.15	2.67 ± 0.84	2.00 ± 0.71	4.00 ± 0.58	4.00 ± 0.00	1.22 ± 0.33

Table 3.3: Each value represents the mean latency (seconds) ± SEM.

3.3.4. EXPERIMENT 1C: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON BEHAVIOUR IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

3.3.4.1. DECREASE IN PRIMARY MOTIVATION: Effects of 1h prefeeding of normal rat chow

During 1hr prefeeding of normal rat chow animals consumed an average of $12.58\text{g} \pm 0.74$. Following feeding, $15.85\text{g} \pm 1.83$ of BW was gained, increasing BW on average by $3.62 \pm 0.28\%$.

3.3.4.1.1. Accuracy of Responding

Fig. 3.7 illustrates the effects of prefeeding of rat chow on accuracy of performance on the task. No significant effect on overall performance was observed following 1hr pre-feeding ($t = -0.575$, $df = 6$, N.S.). Analysis of the independent accuracy during Go and No-go trials further mirrored the lack of effect of accuracy of performance in the task ($t = 0.796$, $df = 6$, N.S.; $t = -1.393$, $df = 6$, N.S., respectively).

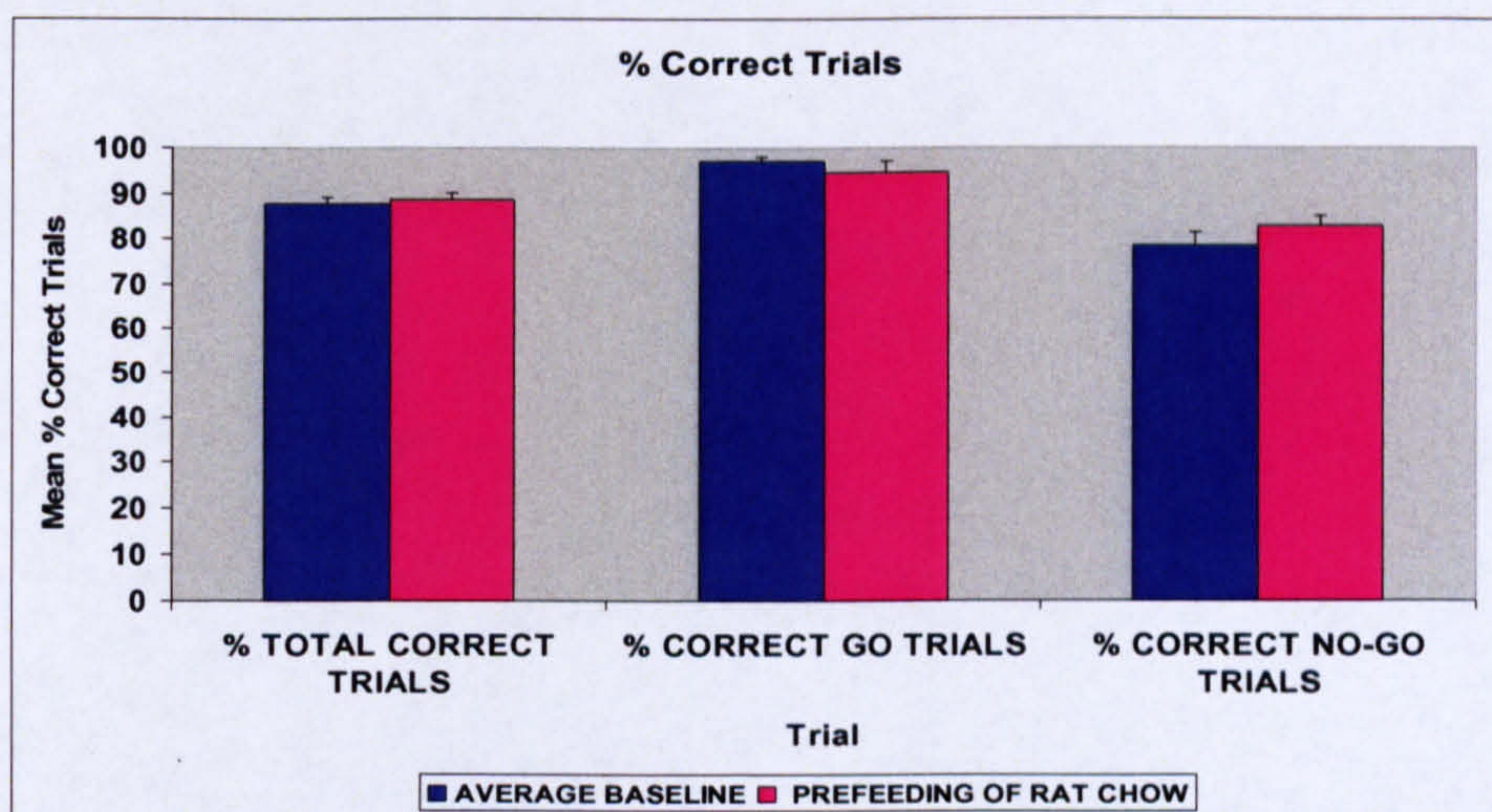


Fig. 3.7: Accuracy of responding: The effects of prefeeding of normal rat chow on percentage correct Total trials, Go trials and No-go trials. Each bar represents the mean percentage \pm SEM.

3.3.4.1.2. Anticipatory Responding

Prefeeding had no effect on early responding during both go ($t = -0.678$, $df = 6$, N.S.) and no go trials ($t = 0.977$, $df = 6$, N.S.). No significant effect of prefeeding was furthermore indicated for inappropriate magazine entries during both Go and No-go trials ($t = 0.858$, $df = 6$, N.S.; $t = 1.208$, $df = 6$, N.S.). Table 3.4 summarises the effects of alterations in primary motivation on anticipatory responding in the task.

3.3.4.1.3. Speed of Responding

Table 3.5 summarises the effects of alterations in primary motivation on speed of responding. Prefeeding of normal rat chow immediately prior to testing had no significant effect on the speed at which animals responded correctly during Go ($t = -1.427$, $df = 6$, N.S.) or incorrectly

during No-go trials ($t = 0.611$, $df = 6$, N.S.).

Latency to collect food rewards during correctly performed Go and No-go trials in addition remained comparable to baseline ($t = 1.427$, $df = 6$, N.S.; $t = -0.932$, $df = 6$, N.S., respectively). Although not significant, as shown in Table 3.5, a trend for magazine latencies to increase was observed following prefeeding.

3.3.4.1.4. Omissions

Failure to collect reward during No-go trials was increased significantly after prefeeding in animals ($Z = -1.997$, $p = 0.046$). (see Table 3.5).

3.3.4.2. DECREASE IN PRIMARY MOTIVATION: Effects of 30mins prefeeding of sucrose reward pellets

During the 30 minutes of prefeeding an average of 12.10 ± 1.03 g of sucrose pellets were consumed. In comparison to the BW immediately prior to feeding, 16.63 ± 2.81 g of weight was gained, increasing BW by $3.69 \pm 0.60\%$.

The amount of normal rat chow and sucrose pellets did not differ significantly across prefeeding manipulations ($t = 0.405$ $df = 5$, N.S). Furthermore, weight gained following each of the prefeeding sessions was comparable, as measured by actual weight gain and percentage increase in weight (all $t \leq -0.301$, $df = 5$, N.S.).

3.3.4.2.1. Accuracy of Responding

Reducing motivation for sucrose pellets had no significant effect on the overall accuracy of performance in the task in comparison to baseline ($t = -1.525$, $df = 5$, N.S). Independent assessment of Go and No-go trials supported further the lack of effect with no significant changes in performance during either Go ($t = -1.470$, $df = 5$, N.S.) or No-go trials ($t = -1.070$, $df = 5$, N.S.). Fig. 3.8 illustrates accuracy of responding in the task following prefeeding of sucrose pellets in comparison to baseline performance.

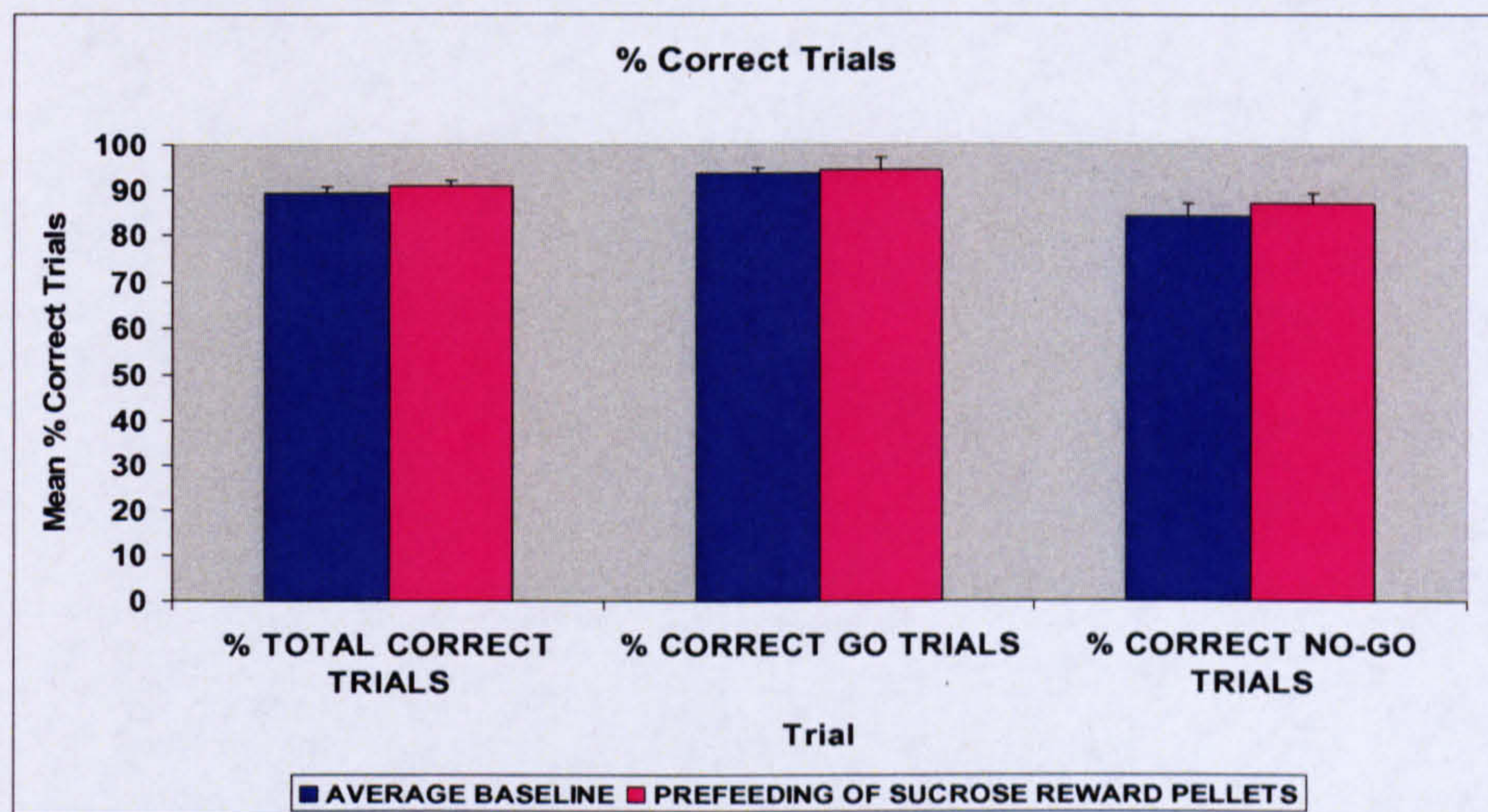


Fig. 3.8: Accuracy of responding: The effects of prefeeding of sucrose reward pellets on percentage correct Total trials, Go trials and No-go trials. Each bar represents the mean percentage \pm SEM.

3.3.4.2.2. Anticipatory Responding

Following the specific decrease in motivation for sucrose reward, approaching significance was a decrease in early responding during both Go ($t = 2.125$, $df = 5$, $p = 0.087$) and No-go trials ($t = 2.127$, $df = 5$, $p = 0.087$) (see Table 3.4). In comparison to baseline performance no significant changes in inappropriate magazine entries were observed during either type of trial (Go trials: $t = 0.246$, $df = 5$, N.S; No-go trials: $t = 2.004$, $df = 5$, N.S.) (see Table 3.4).

3.3.4.2.3. Speed of Responding

Prefeeding of sucrose pellets had no effect on latency to respond either correctly during Go trials or incorrectly during No-go trials ($t = 0.524$, $df = 5$, N.S.; $t = 0.242$, $df = 5$, N.S.) in comparison to BL latency. A non-significant trend for an increase in No-go trials magazine latency was however observed ($t = -1.689$, $df = 5$, $p = 0.076$), whilst no effect on the speed at which reward was collected during Go trials was evident ($t = -0.964$, $df = 5$, N.S.) (see Table 3.5).

3.3.4.2.4. Omissions

Analysis revealed that the frequency of omissions to collect reward during No-go trials significantly increased in comparison to BL following prefeeding of reward pellets ($Z = -2.201$, $p = 0.028$) (see Table 3.5).

3.3.4.3. INCREASE IN PRIMARY MOTIVATION: Effect of reducing allocated food consumption the day prior to testing

Reducing food intake by 50% the day prior to testing, led to an average loss of 5.85 ± 1.03 g of BW, and percentage decrease in weight of $1.30 \pm 0.22\%$.

3.3.4.3.1. Accuracy of Responding

Increasing the level of deprivation had no effect on the total percentage of correct trials performed on the task relative BL accuracy ($t = -0.688$, $df = 6$, N.S.). Independent analysis of Go and No-go trials supported further the lack of influence of decreasing food intake the day prior to testing on task performance ($t = -0.611$, $df = 6$, N.S.; $t = -0.894$, $df = 6$, N.S., respectively) (see Fig.3.9).

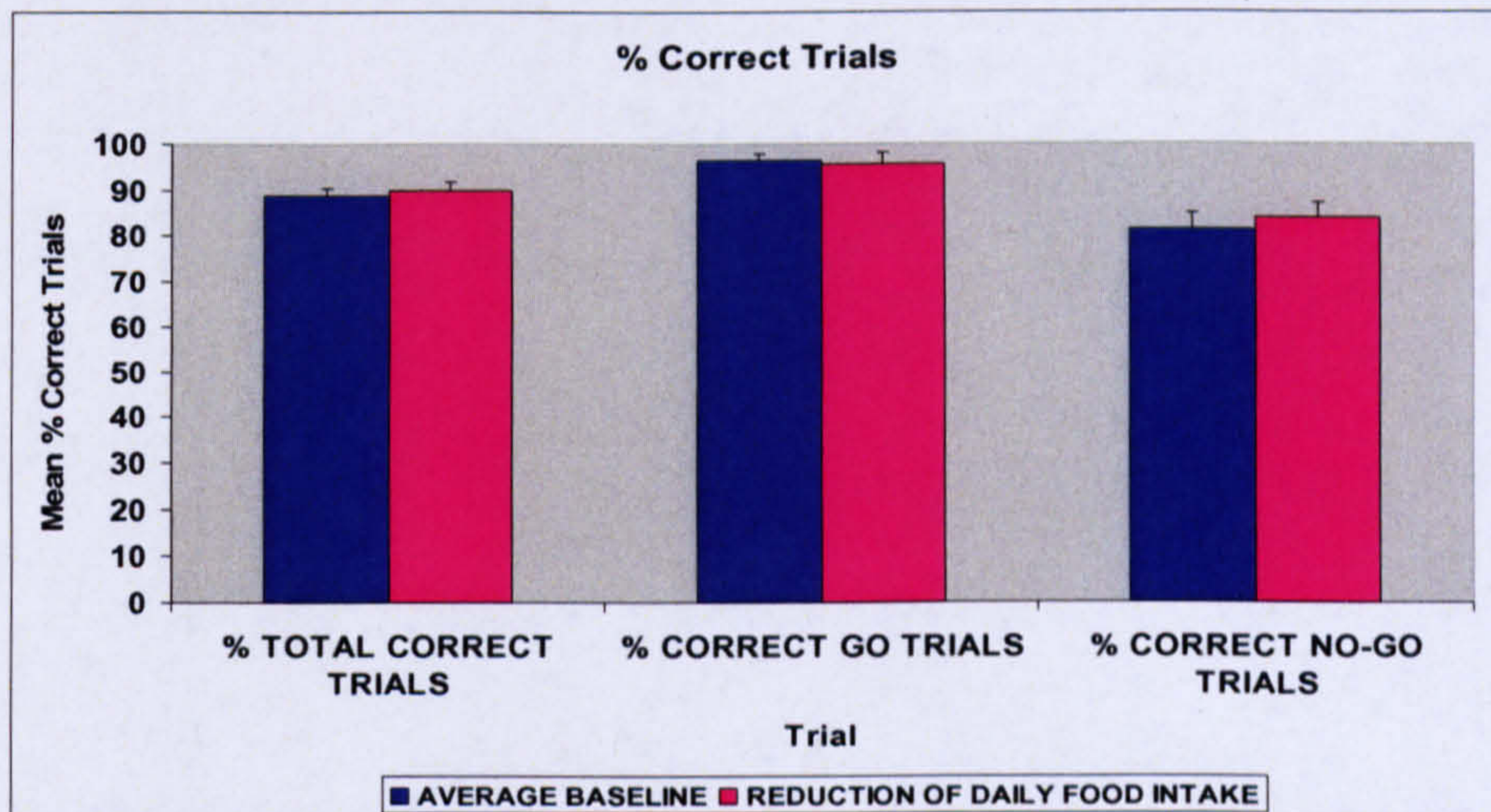


Fig. 3.9: Accuracy of responding: The effects of reducing food intake the day prior to testing on percentage correct Total trials, Go trials and No-go trials. Each bar represents the mean percentage \pm SEM.

3.3.4.3.2. Anticipatory Responding

Early responding during both Go and No-go trials did not differ from baseline following an increase in motivation for food reward ($t = 0.405$, $df = 6$, N.S.; $t = 0.595$, $df = 6$, N.S., respectively). The frequency of magazine entries during Go and No-go trials also remained unchanged ($t = -0.930$, $df = 6$, N.S.; $t = 1.066$, $df = 6$, N.S., respectively) (see Table 3.4).

3.3.4.3.3. Speed of Responding

As shown in Table 3.5 increasing primary motivation decreased significantly the latency to incorrectly respond during No-go trials ($t = 3.526$, $df = 6$, $p = 0.012$). In contrast correct response latency did not differ in comparison to baseline latency ($t = -0.380$, $df = 6$, N.S.).

Furthermore, the speed with which reward was collected following either a correct Go or No-go trial remained unchanged from baseline ($t = 0.516$, $df = 6$, N.S.; $t = -1.655$, $df = 6$, N.S., respectively).

3.3.4.3.4. Omissions

Analysis indicated no effect of increasing deprivation on frequency of failure to collect reward during No-go trials ($Z = -0.211$, N.S.) (see Table 3.5).

Table 3.4: The effects of acute alterations in motivation for food reward on anticipatory responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Average BL Prefeeding of Rat Chow	Prefeeding Rat Chow	Average BL Prefeeding of Sucrose Pellets	Prefeeding Sucrose Pellets	Average BL Reduction of Daily Food Intake	Reduction of Daily Food Intake
Go Trials with Early Responses	16.06 \pm 1.78	14.00 \pm 2.58	15.07 \pm 1.80	10.83 \pm 1.30	14.68 \pm 1.73	14.14 \pm 2.02
No-go Trials with Early Responses	5.09 \pm 1.02	4.29 \pm 0.52	4.73 \pm 1.57	3.17 \pm 1.11	4.14 \pm 0.93	3.86 \pm 0.96
Go Trials with Inappropriate Magazine Entries	2.00 \pm 1.14	2.00 \pm 0.72	1.60 \pm 0.48	1.50 \pm 0.56	2.29 \pm 0.65	3.57 \pm 1.65
No-go Trials with Inappropriate Magazine Entries	12.11 \pm 2.78	10.57 \pm 3.17	11.50 \pm 3.46	7.83 \pm 2.52	12.17 \pm 3.18	9.86 \pm 3.54

Table 3.4: Each value represents the mean frequency \pm SEM.

Table 3.5: The effects of acute alterations in motivation for food reward on speed of responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Average BL Prior to Prefeeding of Rat Chow	Sated Following Prefeeding of Rat Chow	Average BL Prior to Prefeeding of Sucrose Pellets	Sated Following Prefeeding of Sucrose Pellets	Average BL Prior to Reduction of Daily Food Intake	Increased Deprivation Following Reduction of Daily Food Intake
Correct Response Latency	1.01 ± 0.18	1.26 ± 0.21	1.11 ± 0.19	1.20 ± 0.23	1.22 ± 0.23	1.29 ± 0.29
Incorrect Response Latency	2.49 ± 0.26	2.31 ± 0.41	2.55 ± 0.42	2.36 ± 0.58	2.49 ± 0.32	1.54 ± 0.29*
Go Magazine Latency	0.27 ± 0.03	0.30 ± 0.03	0.28 ± 0.03	0.29 ± 0.03	0.27 ± 0.03	0.26 ± 0.03
No-go Magazine Latency	0.61 ± 0.07	0.65 ± 0.08	0.65 ± 0.13	0.79 ± 0.09	0.64 ± 0.11	0.75 ± 0.13
No-go Trial Magazine Omission	0.36 ± 0.14	1.33 ± 0.14*	1.07 ± 0.58	3.00 ± 0.51	0.60 ± 0.14	0.57 ± 0.30

Table 3.5: Each value represents the mean latency (seconds) ± SEM. *, p<0.05, **, p<0.01, *, p<0.001 (Bonferroni comparison) as compared to baseline.**

3.3.5. EXPERIMENT 1D: EXAMINATION OF THE EFFECTS OF VARIABLE NO-GO STIMULUS DURATION ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

3.3.5.1. Accuracy of Responding

Figure 3.10 (a-c) illustrate the effects of varying stimulus duration during no-go trials on accuracy of responding within the task in comparison to BL performance. Varying stimulus duration had no significant effect on overall accuracy of performance in the task in comparison to BL ($t = 1.504$, $df = 5$, N.S.). Independent analysis of Go trials revealed a trend for an improvement in accuracy during the manipulation in comparison to BL. This observation however failed to reach significance ($t = -2.456$, $df = 5$, $p = 0.058$). In contrast, although a trend for a decrease in accuracy during No-go trials was observed during the 20 second stimulus duration trials, analysis of performance revealed no indication of an effect of varying stimulus duration on accuracy of responding during these trials ($F(3, 15) = 2.699$, N.S.).

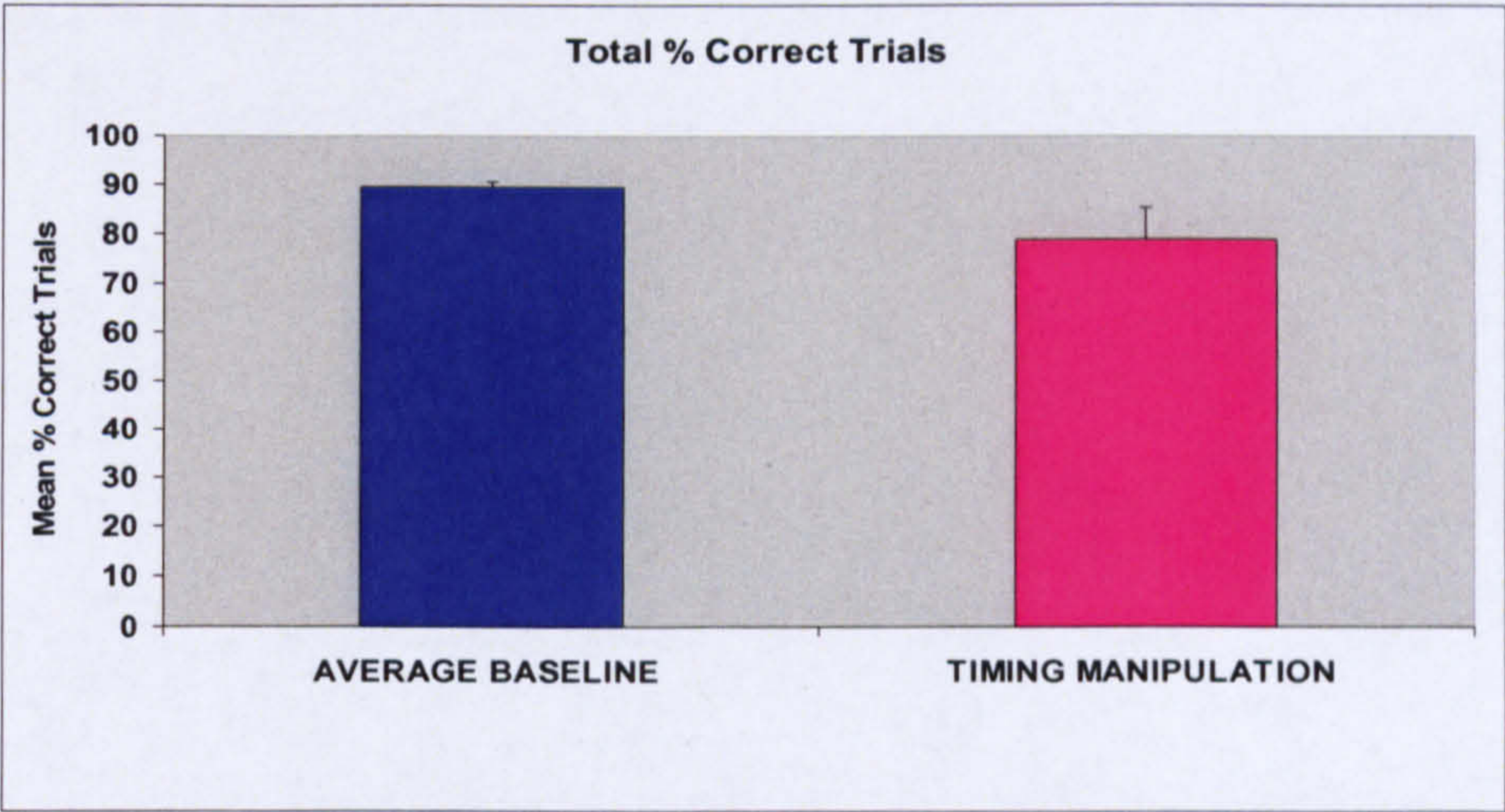


Fig. 3.10 (a)

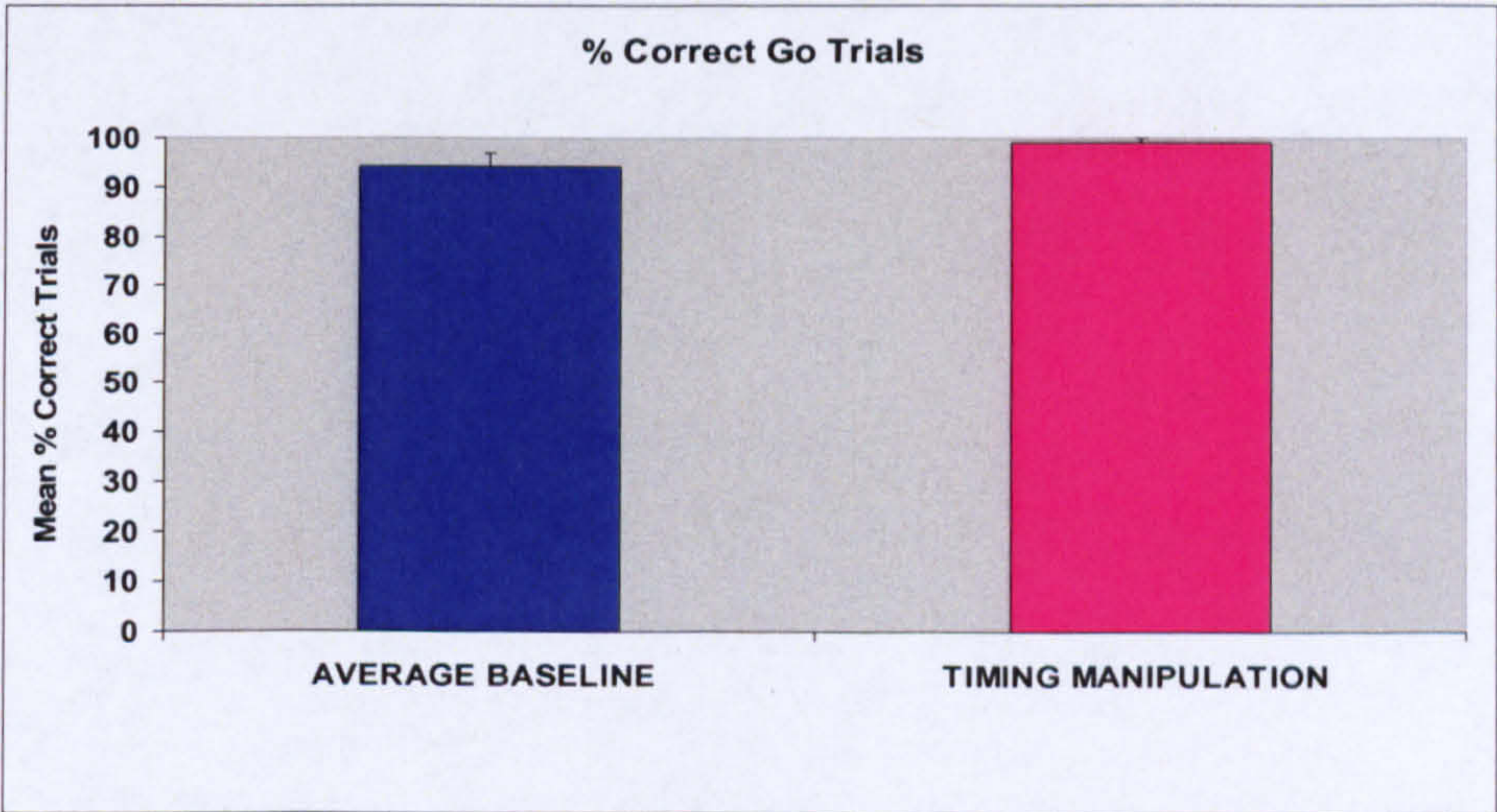


Fig. 3.10 (b)

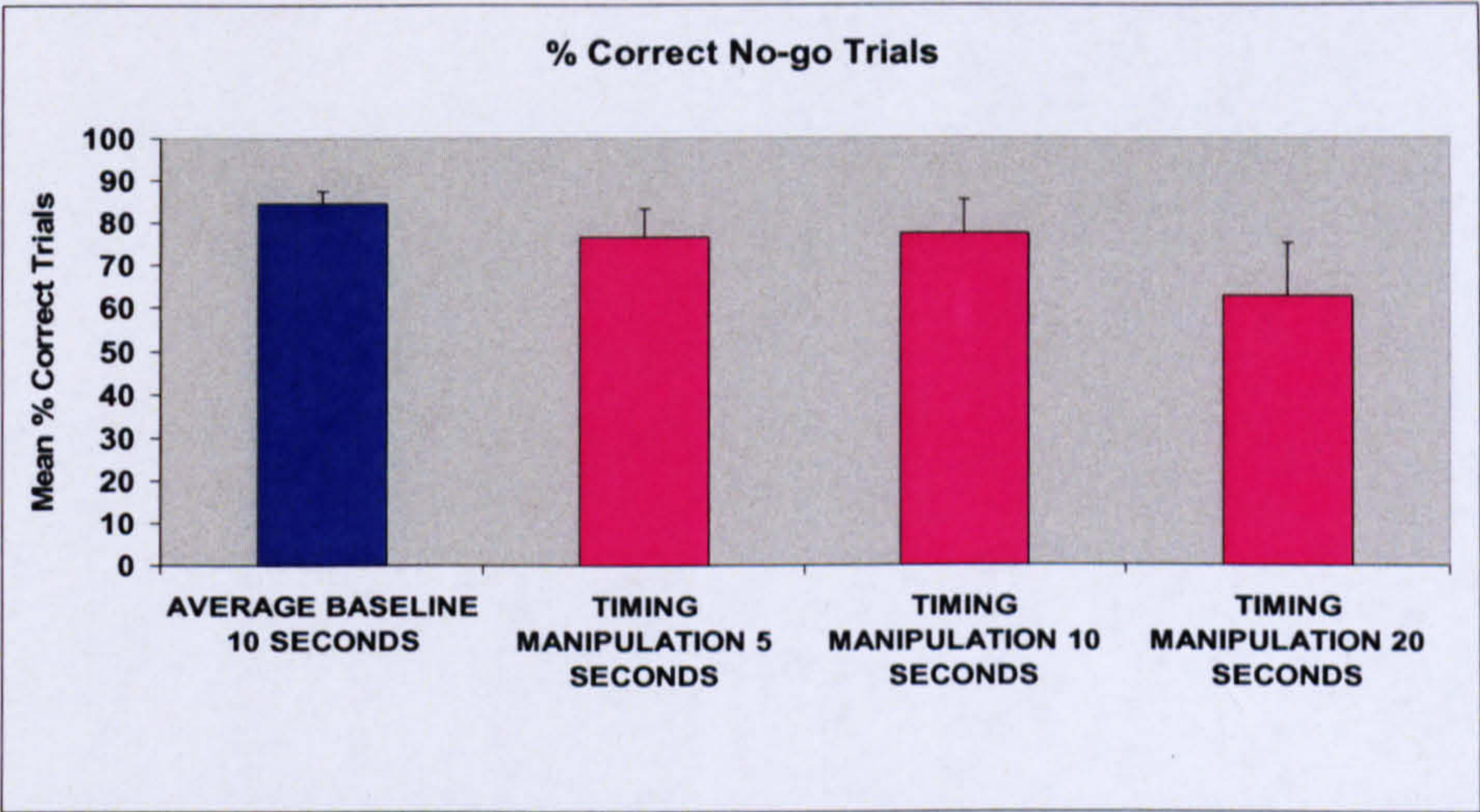


Fig. 3.10 (c)

Fig. 3.10 (a-c): Accuracy of Responding: the effects of varying stimulus duration during No-go trials on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each bar represents the mean percentage \pm SEM.

3.3.5.2. Anticipatory Responding

Variable No-go trial stimulus duration produced no significant effect on frequency of early responding during either Go ($t = -0.630$, $df = 5$, N.S.) or No-go trials ($F(1,5) = 1.559$, N.S.) (Table 3.6). Furthermore, no differences were observed in the frequency of inappropriate magazine entries during Go trials ($t = 0.473$, $df = 5$, N.S.). Conversely, a significant main effect of stimulus duration was observed on inappropriate magazine entries during No-go trials ($F(3,15) = 7.78$, $p = 0.002$). As tabulated in Table 3.6, the proportion of inappropriate magazine entries during stimulus presentation increased with increasing stimulus duration. Post hoc analysis however failed to locate significant differences between trials.

3.3.5.3. Speed of Responding

In comparison to BL performance correct response latency was significantly faster during the manipulation of the task ($t = 3.389$, $df = 5$ $p = 0.019$). Analysis of incorrect response latency however revealed no significant main effect of stimulus duration ($F(3,15) = 1.832$, N.S.) (see Table 3.7)

Varying the stimulus duration during No-go trials led to a significant decrease in magazine latency during Go trials in comparison to BL ($t = -3.548$, $df = 5$, $p = 0.016$). The latency with which reward during No-go trials was collected remained unchanged ($F(3,15) = 0.936$, N.S.) (see Table 3.7).

3.3.5.4. Omissions

Varying stimulus duration had no significant effect on the frequency of omissions to collect reward during no-go trials ($X^2 = 1.909$, $df = 3$, N.S.) (see Table 3.7).

Table 3.6: Anticipatory responding in the symmetrically reinforced go/no-go task following timing manipulation

Behavioural Measure	Average BL 10 seconds	Timing Manipulation 5 seconds	Timing Manipulation 10 seconds	Timing Manipulation 20 seconds
Go Trials With Early Responses	0.34 ± 0.07	-----	0.38 ± 0.33	-----
No-go Trials With Early Responses	0.12 ± 0.03	0.17 ± 0.05	0.11 ± 0.06	0.17 ± 0.06
Go Trials With Magazine Entries	0.38 ± 0.01	-----	0.03 ± 0.01	-----
No-go Trials With Magazine Entries	0.27 ± 0.06	0.18 ± 0.06	0.41 ± 0.10	0.51 ± 0.09

Table 3.6: Each value represents the mean proportion ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to baseline.

Table 3.7: Speed of responding and omissions in the symmetrically reinforced go/no-go task following timing manipulation

Behavioural Measure	Average BL 10 seconds	Timing Manipulation 5 seconds	Timing Manipulation 10 seconds	Timing Manipulation 20 seconds
Correct Response Latency	1.17 ± 0.22	-----	0.86 ± 0.17*	-----
Incorrect Response Latency	2.03 ± 0.33	1.52 ± 0.21	1.90 ± 0.42	2.42 ± 0.48
Go Magazine Latency	0.28 ± 0.22	-----	0.24 ± 0.18*	-----
No-go Magazine Latency	0.68 ± 0.05	0.90 ± 0.26	0.60 ± 0.16	0.78 ± 0.13
No-go Trial Magazine Omission	0.02 ± 0.02	0.08 ± 0.04	0.05 ± 0.05	0.07 ± 0.03

Table 3.7: Each value represents the mean latency (seconds) ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to baseline.

3.4. DISCUSSION

The series of experiments reported in this chapter were designed to test the suitability of the symmetrically reinforced go/no-go paradigm for the exploration of the relationship between drug dependence and impulsivity in later experiments of this thesis. The results demonstrated that task performance is stable and insensitive to changes in primary motivation. Furthermore, accuracy of responding in the task appears to be independent from timing behaviour.

3.4.1. Training and Stability of Task Performance

Animals successfully acquired the task, reaching an 85% overall accuracy of performance. In agreement with previous research, animals exhibited a substantially greater difficulty in learning to inhibit responding during No-go trials (Harrison et al., 1999). Whilst accuracy of responding on Go trials reached above chance level in the first few training sessions, to reach comparable levels of accuracy on No-go trials required approximately 40 training sessions in the majority of subjects. This difference in acquisition across trials can be explained by the CRF lever training the animals received prior to being presented with the conditional stimulus reward contingencies. This training leads to an active response bias, which while favouring performance on Go trials, interferes strongly with learning of the No-go trials. In order to acquire the passive behavioural strategy to obtain reward on No-go trials, animals were first required to inhibit a previously learned response, which was likely to have impeded the learning process.

Once criterion performance had been reached, assessment of performance over a three week period indicated that animals displayed stable overall accuracy of responding in the task, at a level of 85-90% accuracy. Independent performance during Go and No-go trials was furthermore stable across this duration. Other measurable behavioural parameters in the task, such as speed of responding, anticipatory behaviour, and frequency of omissions also displayed no evidence of significant changes across the three week period. Increasing the number of operant sessions to twice per test day had a minimal effect on all behavioural parameters in the task. These findings support, to some extent, previous observations of the stability of behaviour in sham operated control animals (Harrison et al., 1999). Comparable to the present research, accuracy during No-go trials displayed minimal variation over a 20 day period. In contrast to current data, however, is that Go trial accuracy displayed evidence of an improvement over test sessions. Assessment of stability in performance in Harrison et al., (1999) study was, however, conducted in trained animals after a two-week post-operative recovery period. The termination of testing in this study may have led to the lack of stability in performance on these trials.

The establishment of the long-term stability of task performance in the present research is critical for the interpretation of later chronic drug studies of this thesis. Any changes that might

be observed during chronic nicotine administration and nicotine withdrawal can now be more confidently attributed to the effects of the experimental manipulation performed. It is important to highlight that, although stable, accuracy of responding differed across Go and No-go trials. Animals' ability to actively respond during Go trials was almost 100% accurate during task performance, a level of responding that varied minimally across animals. Conversely, the level of accuracy during No-go trials was considerably lower, reaching accuracy of no higher than 80%. These data suggest that, despite extensive training and loss of reinforcement, animals continued to display difficulty in withholding inappropriate responding. The contrasting levels of accuracy during Go and No-go trials is consistent with previous reports of baseline performance on comparable go/no-go tasks in both humans (de Wit et al., 2002) and animals (Fletcher, 1993; Harrison et al., 1999; Paine et al., 2003; Paine and Olmstead 2004). A critical advantage of employing a task where a response prepotency is created that is difficult to inhibit, is that it provides the opportunity to not only observe drug induced deficits in inhibitory control but also improvements in this behaviour (e.g. de Wit et al., 2002).

In further contrast to Go trial performance, was the demonstrated greater variability across animals in the level of performance during No-go trials (see Fig. 3.2(c)). Marked individual differences in levels of inhibitory control have previously been reported in other paradigms of disinhibition, including the 5CSRTT (Dalley et al., 2007) and FCN schedule (Dellu-Hagedorn, 2006). These spontaneous individual differences have led authors to argue that variability in performance across these paradigms may represent differences in 'trait' levels of impulsivity (Robbins, 2002; Dalley et al., 2007).

3.4.2. The Effects of Alterations in Primary Motivation on Task Performance

To ensure, in later studies of this thesis, that the observed effects of nicotine on performance were not governed by drug induced alterations in primary motivation, animals were tested under conditions of varying levels of deprivation. Prefeeding animals normal rat chow immediately prior to the test session had no significant effects on accuracy of responding in the task or on anticipatory behaviour. Whilst speed of responding did not differ significantly from BL, a significant increase in the failure to collect reward during No-go trials was observed. Although magazine latency has traditionally been used to index motivation (Robbins, 2002), task omissions and response latencies have recently been argued to more consistently reflect the level of motivation for reward in animals in paradigms such as the 5CSRTT (Grottick and Higgins, 2002; Bizarro and Stoleran, 2003). It can therefore be argued that motivation for food reward was effectively decreased following prefeeding of normal chow. A comparable behavioural profile was observed following the reduction of specific satiety for sucrose reward. No significant changes were observed on accuracy of responding, anticipatory behaviour or latencies during performance. Once again, supporting the lack of motivation for food reward, a

significant increase in omissions to collect reward during No-go trials was shown. These findings are inconsistent with the reported decrease in anticipatory responding reported in the 5CSRTT following additional food prior to testing (Carli and Samanin, 1992; Harrison et al., 1997; Grottick and Higgins, 2000; Grottick and Higgins, 2002; Bizarro and Stoleran, 2003). The possibility that the prefeeding manipulations of the present research did not reduce motivation to a great enough extent to observe alterations in performance, can not easily account for the discrepancy in findings. In the majority of previous research, animals consumed *less* food (4-5g) prior to testing than animals in the present study (12-12.6g), suggesting that motivation was reduced to a greater extent in the present research. Differences between the paradigms utilised to measure inhibitory control are more likely to explain the variation in findings. Unlike the go/no-go task, the 5CSRTT is primarily a model of sustained attention. It may be the case that the single parameter used to assess impulsive responding in this model is more sensitive to motivational changes than inhibitory measures in the go/no-go task.

Acutely increasing the motivation for food reward also did not affect accuracy of responding or anticipatory behaviour on the task. Whilst the frequency of omissions remained unchanged from BL, evidence of an increase in motivation for food reward was demonstrated by the significantly faster speed with which incorrect responses were made during No-go trials. The suggestion that disinhibited responding, as assessed by the 5CSRTT, is enhanced following an increase in motivation (Bizarro and Stoleran, 2003), is therefore largely unsupported by the present findings. The different paradigms utilised across studies, and the more severe level of deprivation induced in previous research may have accounted for the discrepancy in findings.

Taken together, the lack of effect of both prefeeding and increasing deprivation on both accuracy of responding during No-go trials and anticipatory responding, suggest that measures of inhibitory control in this task are insensitive to changes in primary motivation. It can therefore be argued that it is unlikely that either the anorectic effects of nicotine or increased appetite associated with nicotine withdrawal will mediate any nicotine induced changes in inhibitory control that may be observed (Grunberg 1982; Grunberg et al., 1986; Klesges et al., 1989; Miyata et al., 1999; Zhang et al., 2001).

3.4.3. Timing Behaviour

A factor that has hindered the interpretation of the effects of drugs of abuse in animal models of disinhibition, such as the DRL, is that performance in such tasks is highly dependent on timing ability (Paule et al., 1999; Wiley et al., 2000). As timing mechanisms are known to be sensitive to a number of drugs of abuse, including nicotine (Carrasco et al., 1998), it was essential to demonstrate that performance in go/no-go task was not heavily dependent on time perception. Findings revealed that varying the duration of stimulus presentation during No-go trials did not

affect overall accuracy, or independent performance during both Go and No-go trials in the task. Altering the duration of stimulus presentation did, however, lead to alterations in frequency of inappropriate magazine entries during No-go trials. Inappropriate magazine entries were found to increase with longer stimulus duration. Post hoc analysis, however, failed to locate significant differences in comparison to the frequency of responding during BL. The time in which animals correctly responded and collected the reward during Go trials did, however, become significantly faster in comparison to BL performance. Conversely, varying the duration of stimulus presentation during No-go trials had no effect on the speed of responding during these trials. These findings suggest that the primary measures of disinhibition in the task (No-go trial performance); is not dependent on timing mechanisms, as supported by the comparable level of accuracy of responding during trials of varying stimulus duration. Accuracy of responding in the task therefore appears to be highly conditioned to exteroceptive cues in the task, which minimises the need for timing behaviour. Animals appear to be utilising the differing visual cues to inform them when to respond and when not to respond (Harrison et al., 1999). The findings of the present study importantly indicate that nicotine induced changes on this measure of inhibitory control are unlikely to be related to alterations in timing processes. However, the evident increase in inappropriate magazine entries during No-go trials suggests that caution may need to be given when interpreting the effects of nicotine on this parameter. This behaviour appears to be more sensitive to drug induced effects on timing mechanisms, although it should be stressed that the alterations observed on this anticipatory measure did not differ significantly from BL. The decreased speed of responding during Go trials is difficult to interpret as these trials during the manipulation remained unchanged from the normal task procedure. The number of Go trials was however reduced to 10 trials during the manipulation. The observed faster speed of responding may have therefore reflected a heightened anticipation of eagerly awaited Go trials.

3.4.4. Limitations

Two potential limitations of the studies in this chapter should be noted. Firstly, due to illness, several animals had to be removed from study, reducing the N to 6 for the majority of experiments of this chapter. It is possible that the small subject number could have led to a decrease in power to detect an effect (type II error). However, with the exception of Experiment 1D, no critical trends in the data were observed that failed to reach significance, suggesting that inclusion of a greater number of animals would have been unlikely to have changed dramatically the main conclusions of the present chapter.

Secondly, whilst acute manipulations of primary motivation effectively compare to later studies that investigate the acute administration of nicotine, longer term alterations in levels of motivation would have more effectively compared to future long term drug studies where

chronic nicotine administration and withdrawal will be investigated. Therefore, in subsequent studies of this thesis that assessed the appropriateness of the delayed reward task, investigation was also made of the prolonged effects of alterations in motivation for food reward on task performance.

3.4.5. Conclusions

In summary, the results of the present chapter have confirmed the suitability of the symmetrically reinforced go/no-go task as a paradigm that will enable the investigation of both acute and long term nicotine administration and nicotine withdrawal. Clear demonstration has been made of the long term stability of task performance and lack of sensitivity of behavioural disinhibition to changes in primary motivation. Finally, the presence of extroceptive cues in the task appears to minimise greatly the need for timing behaviour for accurate responding during No-go trials. Caution however, may need to be given when interpreting the effects of nicotine on inappropriate magazine entries.

CHAPTER 4

Acute effects of nicotine on behavioural disinhibition and the mediating role of the central nicotinic receptors

4.1. INTRODUCTION

4.1.1. General Introduction

The loss of inhibitory control is believed to be a key component of drug addictive disorders that has been attributed primarily to dysfunction of the PFC (Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004). The impaired behavioural disinhibition in drug dependent individuals has been proposed to underlie both the inability to regulate compulsive drug use and maintain successful abstinence despite the devastating health consequences (Goldstein et al., 2004; Lubman et al., 2004).

Utilising behavioural measures of inhibitory control, past research has demonstrated extensive support for the heightened impulsive responding across different populations of drug abusers, including cigarette smokers (e.g. Fillmore and Rush, 2002; Spinella, 2002; Forman et al., 2004; Li et al., 2006). On tasks such as the CPT and the go/no-go paradigm, current heavy smokers have been shown to display a poorer ability to withhold inappropriate responding (Spinella, 2002; Dinn et al., 2004; Yakir et al., 2007). Whilst such research strongly supports the association between smoking and disinhibition, the research does not address the key issue of whether disinhibition is a consequence of drug use or a risk factor in the development of nicotine dependence. As previously discussed, one way to further our understanding of the causal relationship between drug dependence and disinhibition is to assess the acute effects of nicotine on tasks designed to measure disinhibition.

Although relatively few studies have attempted this, results to date have been inconsistent. No significant effects of acute nicotine on inhibitory control, as assessed by the CPT and SST, were reported in both non-smokers and overnight abstinent smokers (Levin et al., 1998; Bekker et al., 2005a). Conversely, amongst neuropsychiatric populations suffering from ADHD and schizophrenia, where baseline inhibitory control is abnormally low, acute nicotine *increased* inhibitory control (Levin et al., 1996b; Potter and Newhouse, 2004). It has been suggested that the positive effects of acute nicotine on behavioural inhibition may explain the high prevalence of smoking in these populations (de Leon et al., 1995; Pomerleau et al., 1995; Dervaux et al.,

2004). If true, this theory would suggest that impairments in inhibitory control may perhaps predispose individuals to initiate smoking.

In the animal literature experiments assessing the acute effects of nicotine have primarily focused on attentional tasks (Carli et al., 1983). Acute nicotine treatment increased anticipatory responding in the 5CSRTT, only at low doses (0.03-0.3mg/kg) and under task conditions of high attentional demand (e.g. Mirza and Stoleran, 1998; Blondel et al., 2000; Stoleran et al., 2000; Mirza and Bright, 2001; Hahn, et al., 2002; Hahn et al., 2003; Bizarro, et al., 2004; Bruin et al., 2006; Day et al., 2007). An increase in inappropriate premature responding during a DRL schedule of reinforcement has also been reported following the acute administration of nicotine (Morrison, 1968; Bizot 1998; Popke, Mayorga, Fogle and Paule 2000a; 2000b). However, this effect may be related to nicotine's known effects on time perception (e.g. Hinton and Meck 1996; Carrasco et al., 1998).

Although behavioural studies have yielded inconsistent and unconvincing data, nicotine is known to alter the functioning of neurobiological mechanisms involved in the mediation of inhibitory control (Kirch et al., 1987; Vezina et al., 1992; Ramussen and Czachura, 1995; Nisell, 1996; Balfour et al., 1998; Hildebrand et al., 1998; Harrison et al., 1999; Hildebrand, Panagis, Svensson and Nomikos, 1999; Olausson et al., 2001; de Wit et al., 2002; Christakou et al., 2004; Rahman et al., 2004; Picton et al., 2007). Pharmacologically, nicotine initiates its agonistic actions by binding to the nicotinic acetylcholine receptors (nAChRs) that are located throughout the central (CNS) and peripheral nervous system. Most relevant to nicotine addiction are the neuronal nAChRs of which twelve subunits have been identified, α 2-10 and β 2-4 (Benowitz, 1996; Role and Berg, 1996; Salamone and Zhou, 2000). The nAChRs are pentameric combinations of the α and β subunits, the most common formed in the brain being the heteromeric α 4 β 2 and the homomeric α 7, accounting for 85% and 10% of the total nicotinic receptors respectively (Seguela et al., 1993; McGlee and Role, 1995; Zoli et al., 1998; Lukas et al., 1999). Nicotine receptors are present in several brain regions including the ventral tegmental area (VTA), striatum, NAc, PFC, amygdala and septal area (Clarke et al., 1985; Wada et al., 1989; Marks et al., 1992; Nayak et al; 2000; Woolterton et al., 2003). In the CNS nicotine, via presynaptic and postsynaptic nAChRs, influences the release of various neurotransmitters including acetylcholine, noreadrenaline, DA, 5-HT, glutamate and GABA (Wonnacott et al., 1990; Wonnacott, 1997; Li et al., 1998; Jones et al., 1999).

4.1.2. Objectives of Experiment 2 (A-C)

The primary objectives of Experiment 2 were therefore:

- i) To assess the acute effects of nicotine on disinhibition as measured by the symmetrically reinforced go/no-go visual discrimination task.
- ii) To determine whether the effects of acute nicotine on disinhibition are mediated through central nAChRs.

To achieve these objectives a dose response function for nicotine was initially determined on behavioural disinhibition using the go/no-go task. To establish whether nicotine's effects on performance were mediated by central nicotinic receptors, a dose response function for mecamylamine (MEC) was also established. MEC is an effective non-specific antagonist of the central nicotinic receptors. The classic antagonist is generally believed to be non-competitive in its action although it has been proposed that MEC may also exhibit some competitive properties (Varanda et al., 1985; Martin, 1989; 1990; Francis and Papke, 1996). Finally, having determined effective doses of nicotine and 'silent' doses of MEC on task performance, combination drug treatments of nicotine and MEC were administered to assess whether MEC could antagonise the effects of nicotine in the go/no-go task.

4.2. EXPERIMENT 2 (A-C): EXAMINATION OF THE ACUTE EFFECTS OF NICOTINE ON BEHAVIOURAL DISINHIBITION AND THE MEDIATING ROLE OF THE CENTRAL NICOTINIC RECEPTORS

4.2.1. METHOD

4.2.1.1. Subjects

Subjects were 12 adult male Lister Hooded rats (Charles River, UK). On arrival animals were housed in pairs and maintained under a 12 hour light/dark cycle (lights on at 0700h) in a controlled environmental temperature of $21\text{ }^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and relative humidity of $50\% \pm 10\%$. Animals weighed approximately 300-350g at the start of testing and were maintained at 85% of their free feeding adult body weights throughout the testing period. Water was available ad libitum in home cages and feeding occurred at the end of each experimental day. The same subjects were utilised for Experiments 2(A-C). However, following completion of Experiment 2A one subject was removed from study due to illness. A total of 11 subjects were therefore used in experiments 2B and 2C. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996.

4.2.1.2. Apparatus

Four identical operant chambers were used (30.5 X 24.1 X 29.2cm Med Associates Inc., USA). See Chapter 2 section 2.5.1 for detailed description of apparatus.

4.2.1.3. Behavioural Testing

Pre-training and the behavioural procedure of the symmetrically reinforced go/no-go conditional visual discrimination task have been outlined in detail previously in the methodology of Chapter 3, section 3.2.1.3. Subjects were trained for approximately 8 weeks until performance had stabilised at 85% total correct trials or above. During training animals could conduct the behavioural task up to twice per day.

4.2.1.4. Drugs

Both nicotine hydrogen tartrate salt and mecamylamine hydrochloride (MEC) were dissolved in 0.9% saline and administered s.c. in a volume of 1ml/kg body weight. All doses were calculated as free base. The pH of all nicotine drug solutions was adjusted to approximately 6, using 0.1M sodium hydroxide. Nicotine (0, 0.125, 0.25, 0.5 and 1.0mg/kg) was administered s.c. 10 minutes prior to testing. Mecamylamine hydrochloride (0, 0.1, 0.3 and 1.0mg/kg) was administered s.c. 20 minutes prior to operant testing.

4.2.1.5. Design and Procedure

Experiments 2(A-C) employed a within-subjects design. The doses for each experiment were administered according to a Latin square design with a minimum of 72 hours between the administration of consecutive treatment doses. It was essential that animals had returned to baseline performance prior to the subsequent treatment condition being administered. Baseline performance was defined as behaviour in the task deviating no greater than 5% from the accuracy of performance reached by the subject prior to initiating drug treatment. This drug regime was adhered to in all experiments. A minimum of a one week 'wash out' period was given between different drug experiments to minimise the possibility of drug carry over effects. During the seven day drug free period animals continued to perform the task daily. In the week prior to drug testing, animals were habituated to injection procedures, with subjects s.c. injected with 1ml/kg saline 10 minutes prior to the operant session. Each experiment followed standard operant testing procedures, outlined in detail in general methodology of Chapter 2, section 2.5.2. Injection procedures, during all experiments, were conducted in a procedure room separate from both the holding room and operant test laboratory. All testing took place during the light phase of their LD cycle between 1500h and 1800h. Experimentation was conducted over an 8 week period.

4.2.1.5.1. EXPERIMENT 2A: THE EFFECTS OF ACUTE ADMINISTRATION OF NICOTINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Initially the dose-response function of nicotine (0, 0.125, 0.25, 0.5 and 1.0mg/kg, s.c.) was first examined in the symmetrically reinforced go/no-go task. Following drug administration animals

were transferred immediately to the operant test room.

4.2.1.5.2. EXPERIMENT 2B: THE EFFECTS OF ACUTE ADMINISTRATION OF MECAMYLAMINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Following Experiment 2A the MEC dose-response (0, 0.1, 0.3 and 1.0mg/kg) on the symmetrically reinforced go/no-go paradigm was assessed. In contrast to experiment 2A, due to the longer inter-injection interval for the antagonist, animals were first returned to their holding rooms until 10 minutes prior to testing, at which point they were transferred directly to the operant testing laboratory.

4.2.1.5.3. EXPERIMENT 2C: THE EFFECTS OF CO-ADMINISTRATION OF NICOTINE AND MECAMYLAMINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Following Experiment 2B nicotine and MEC were co-administered to determine whether the nicotine induced disinhibition was in part mediated by central nicotinic receptors. The 0.5mg/kg dose of nicotine was selected on the basis that this dose significantly increased behavioural disinhibition in the task during Experiment 2A. Mecamylamine dose-response data from experiment 2B indicated a lack of disruption of performance in the task across the dose range tested, enabling three 'silent' doses 0.1, 0.3 and 1.0mg/kg of the antagonist to be co-administered with nicotine. Treatment conditions during the Experiment 2C were saline/saline, saline/NIC_{0.5mg/kg}, MEC_{0.1mg/kg}/NIC_{0.5mg/kg}, MEC_{0.3mg/kg}/NIC_{0.5mg/kg} and MEC_{1.0mg/kg}/NIC_{0.5mg/kg}. The saline/saline and saline/ NIC_{0.5mg/kg} drug combinations both served as treatment controls. The first of these, saline/saline, controlled for the effects of combination injections on behaviour in the task, whilst the combined administration of saline/ NIC_{0.5mg/kg}, acted as a positive drug control. The first of all combination treatments was administered 20 minutes prior to the testing session, following which animals were returned to their holding room. Ten minutes prior to testing, animals were then returned to the preparation room where the second treatment was administered after which they were transferred directly to the operant testing room.

4.2.1.6. Statistical Analysis

For all drug experiments behavioural measures, including accuracy of responding, anticipatory responding, speed of responding and omissions were analysed using a one-way repeated measures ANOVA, with treatment dose as the within subject factor. Significant main effects were investigated further by post hoc Bonferroni pairwise comparisons.

Accuracy of responding on the task during non-drug test days was additionally compared across Experiments 2A-C. This comparison was conducted to determine whether changes in baseline accuracy of performance took place during the eight weeks of drug testing. Independently for

each experiment, the accuracy of performance displayed during the baseline sessions immediately prior to each treatment dose was averaged. The average baseline accuracy for Experiment 2A, 2B and 2C was then analysed by a one-way repeated measures ANOVA with Experiment as the within subject factor, followed where appropriate by Bonferroni comparisons.

In cases where sphericity was violated the Greenhouse-Geisser correction was applied and the degrees of freedom adjusted accordingly to more conservative values. Data that violated normality was subjected to the appropriate transformations (see section 2.7). If data could not be successfully transformed, then the non-parametric Friedman test was employed, followed where appropriate by Wilcoxon Signed Ranks tests. In all cases of analysis α values of $p < 0.05$ were deemed statistically significant.

4.3. RESULTS

Further supplementary statistical information (ANOVA tables) can be found in Appendix 1. Magazine omissions (the failure to collect reward) rarely occurred throughout drug experimentation and therefore analysis of this behaviour was not conducted.

4.3.1. Baseline Performance

Baseline accuracy of performance increased over sessions, leading to a significant difference in overall percentage correct trials across experiments ($F(2,20) = 11.442, p < 0.001$) (Fig. 4.1). Post hoc analysis demonstrated that baseline accuracy increased significantly during Experiments 2B and 2C in comparison to performance during Experiment 2A (all $p < 0.05$). Independent assessment of Go and No-go trials revealed that the increase in accuracy of performance across experiments was due to enhanced performance during No-go trials during experiments 2B and 2C ($F(2,20) = 12.403, p < 0.001$). Baseline performance on Go trials in contrast did not differ across drug experiments ($F(2,20) = 0.248, N.S.$).

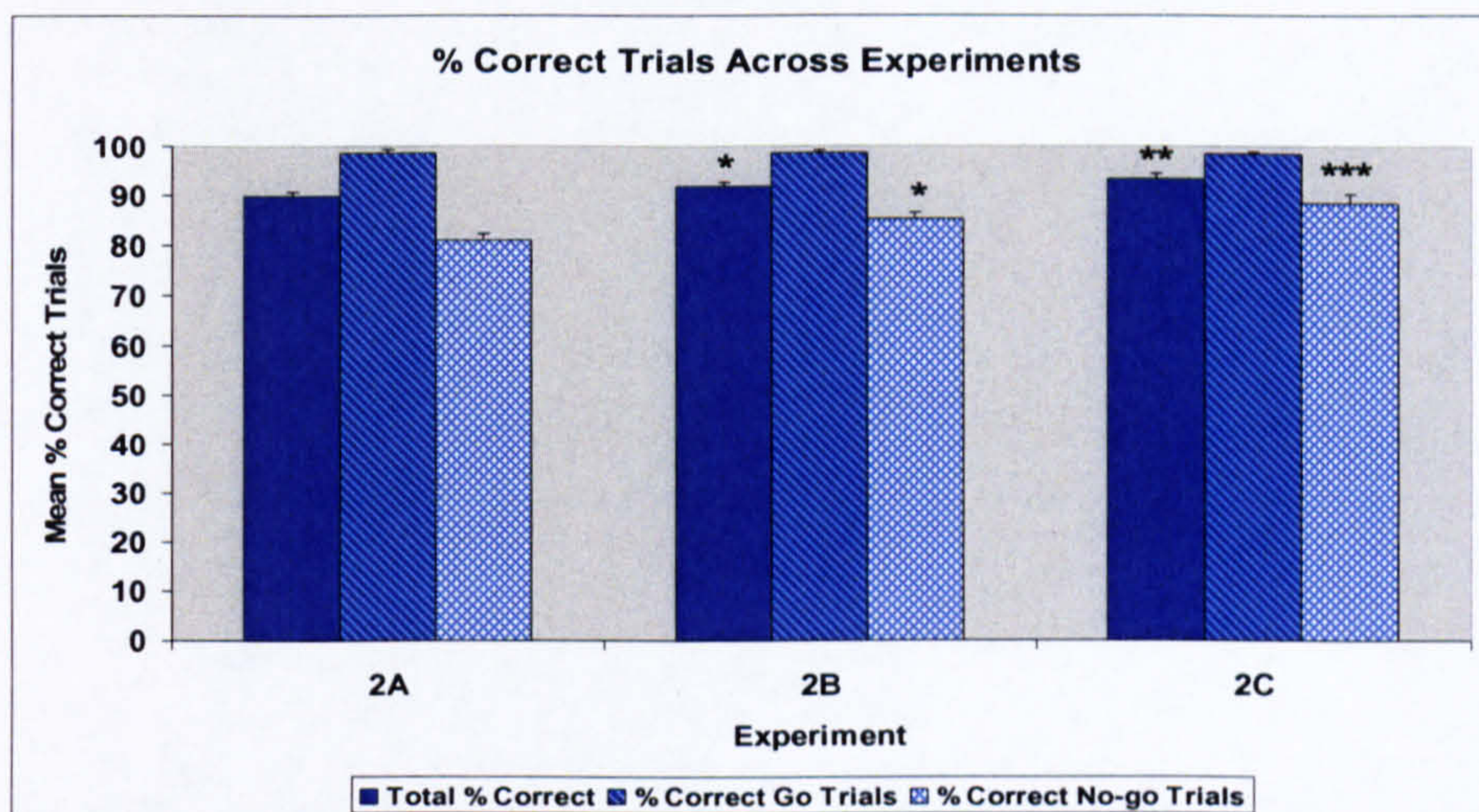


Fig 4.1: Comparison of baseline accuracy of performance of Experiments 2A-2C. Each bar represents the mean percentage correct trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to performance during Experiment 2A.

4.3.2. EXPERIMENT 2A: THE EFFECTS OF ACUTE ADMINISTRATION OF NICOTINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

4.3.2.1. Accuracy of Responding

Acute administration of nicotine produced a significant deficit in overall performance in the task ($F(4,44) = 5.769$, $p = 0.001$) (Fig. 4.2(a)). Bonferroni comparisons demonstrated a significant difference between saline control and the 0.5mg/kg ($p < 0.05$) and highest 1.0mg/kg ($p < 0.05$) doses of nicotine.

Independent assessment of accuracy of performance on Go and No-go trials revealed a significant reduction in accuracy during both trial types following nicotine treatment ($F(4,44) = 12.264$, $p < 0.001$; $F(4,44) = 2.802$, $p = 0.037$, respectively). As illustrated in Fig 4.2 (c), post hoc comparisons indicated an inability to withhold a response on No-go trials after 0.5mg/kg of nicotine ($p < 0.05$). In contrast, performance during Go trials was reduced following a dose of 1.0mg/kg of nicotine (Fig. 4.2 (b)). The highest dose differed not only from saline but also from the performance on Go trials following treatment with 0.125mg/kg and 0.25mg/kg of nicotine (all $p < 0.01$).

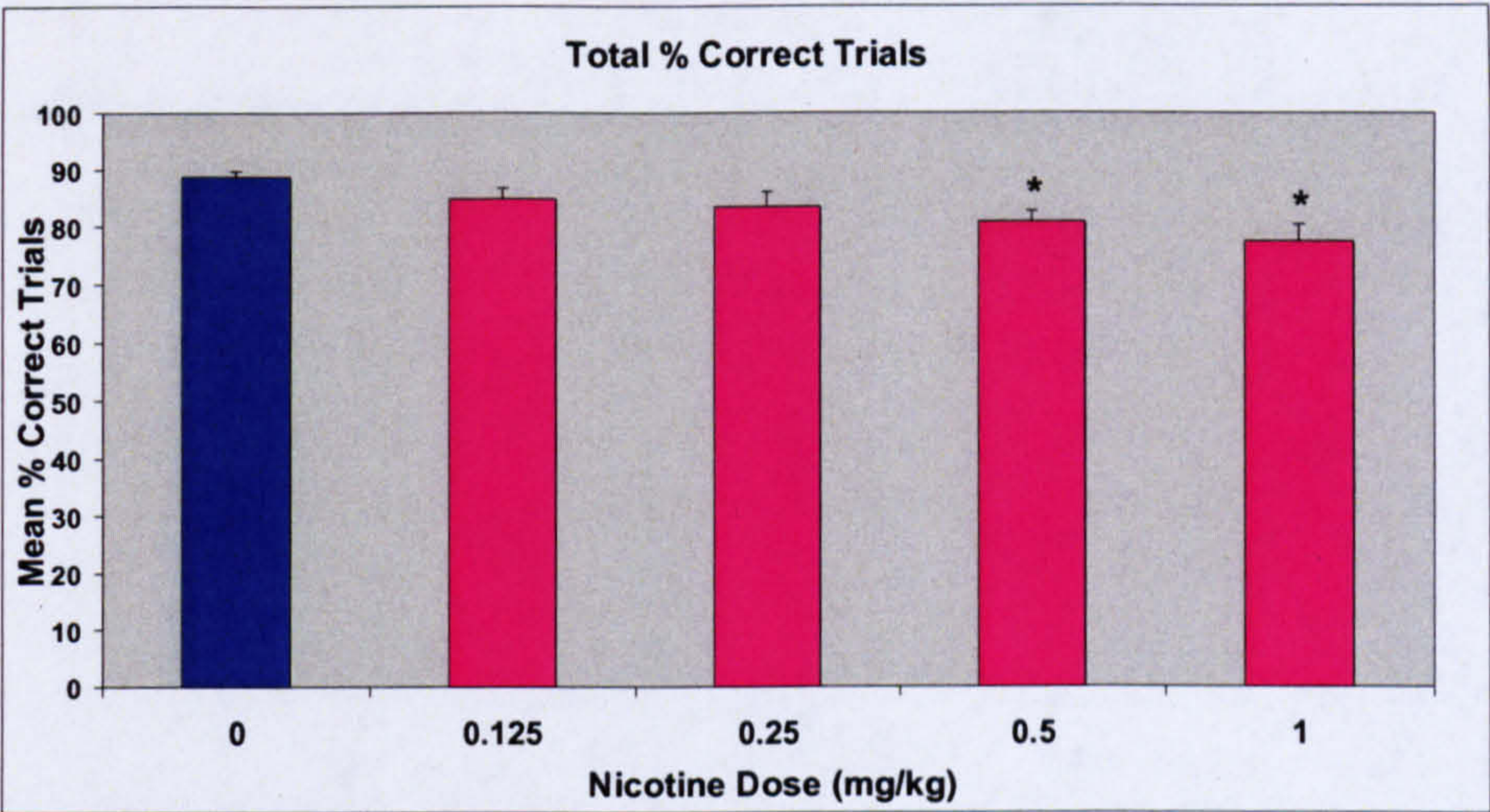


Fig. 4.2(a)

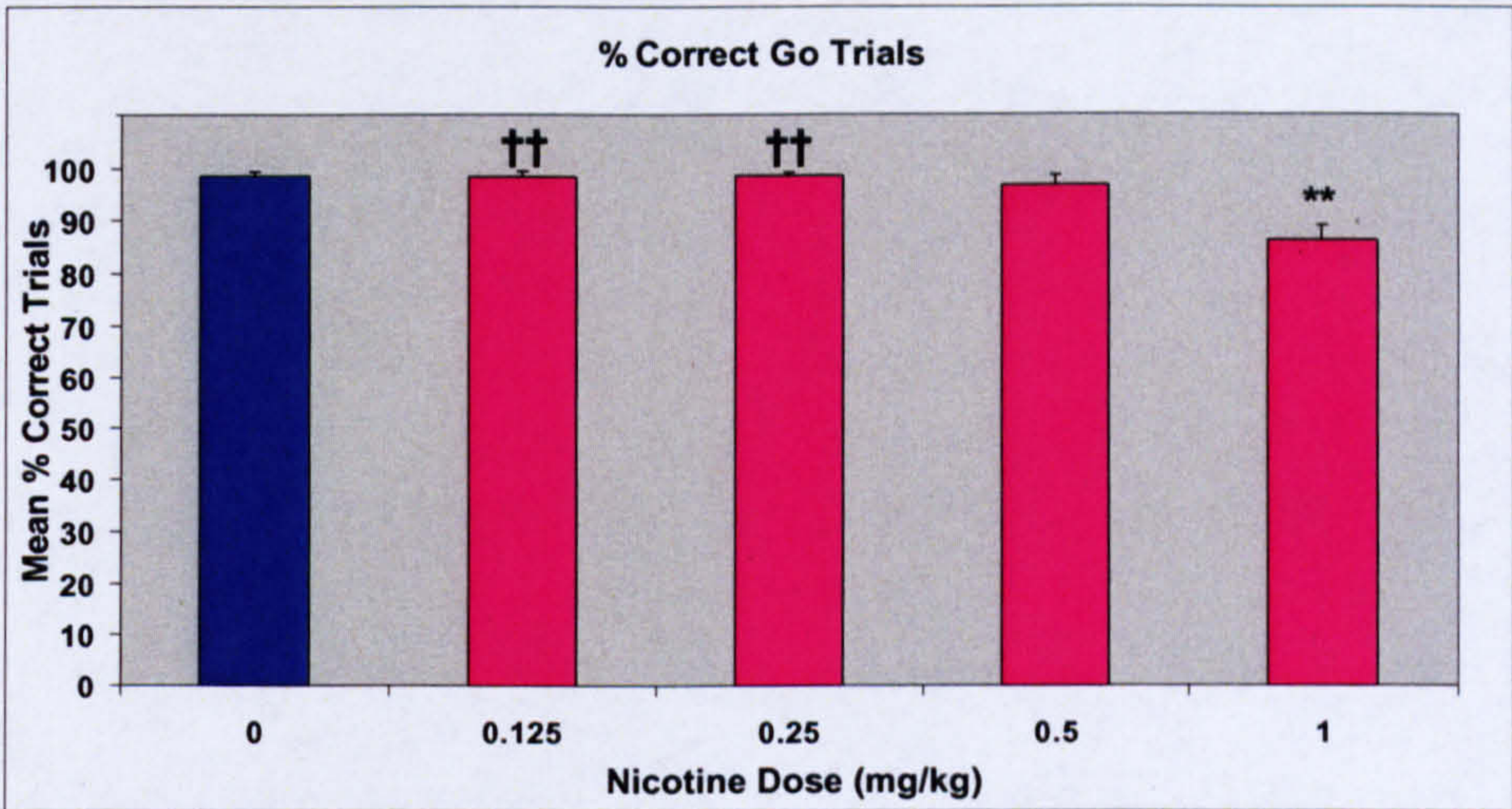


Fig. 4.2(b)

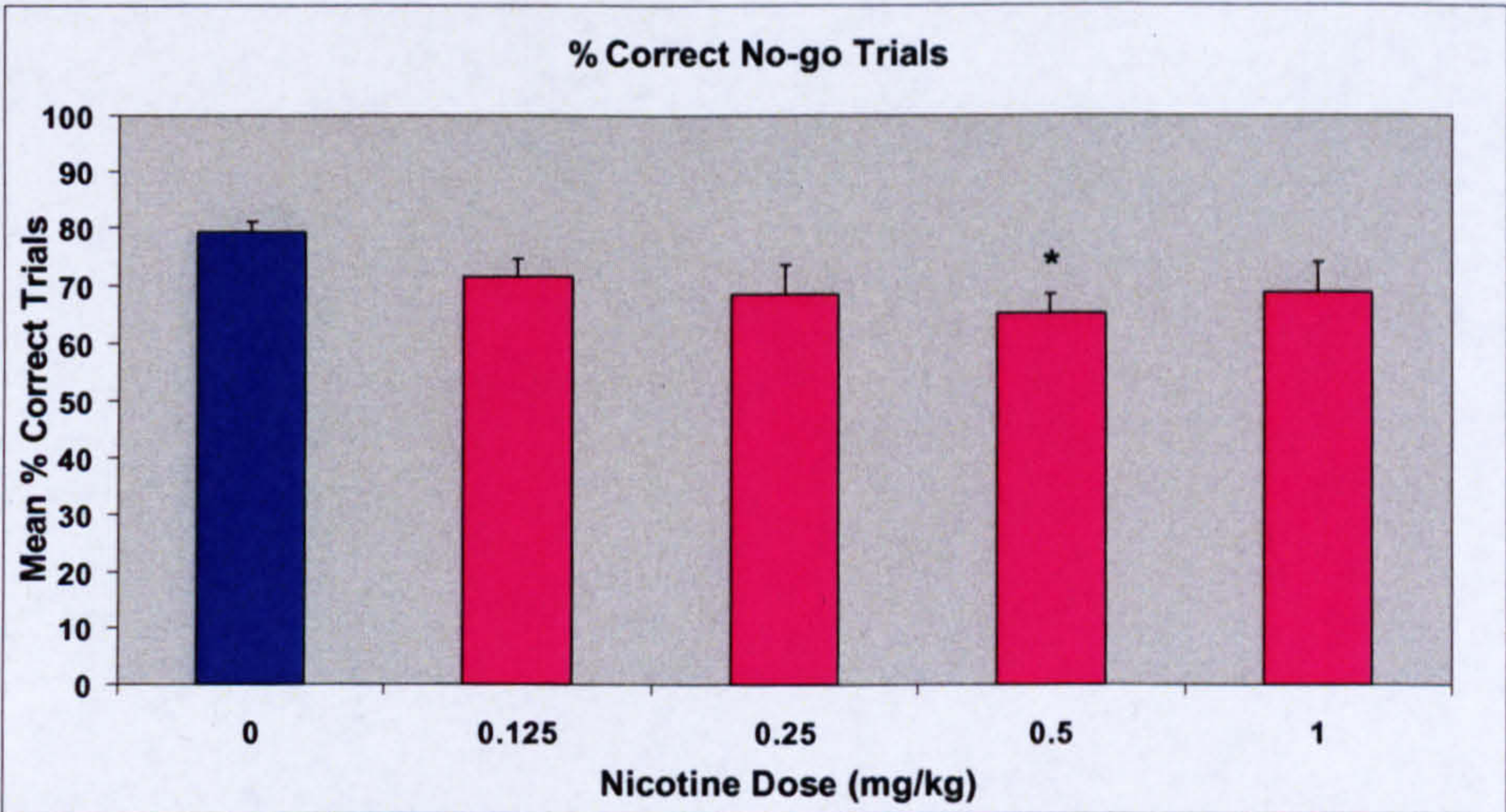


Fig. 4.2(c)

Fig. 4.2 (a-c): Accuracy of Responding: the effects of acute nicotine administration on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each bar represents the mean percentage correct trials \pm SEM. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ (Bonferroni comparison) as compared to saline control. †, $p<0.05$, ††, $p<0.01$, †††, $p<0.001$ (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

4.3.2.2. Anticipatory Responding

Figure 4.3 (a-d) illustrate the effects of acute nicotine treatment on anticipatory responding in the behavioural task. With the exception of the highest dose, nicotine treatment increased the frequency of early responses during trials. This trend reached significance during Go trials where a main effect of drug treatment was found ($F(4,44) = 9.368$, $p < 0.001$) (Fig 4.3 (a)). Post hoc analysis revealed that following treatment with 0.125mg/kg the frequency of early responses significantly increased in comparison to the control and the highest dose of nicotine (all $p < 0.05$). Furthermore, at the highest 1.0mg/kg dose, the decrease in early responding during the pre-discrimination period reached significance in comparison to 0.5mg/kg nicotine ($p < 0.01$). Analysis of this anticipatory response measure during No-go trials also confirmed a main effect of treatment ($F(4,44) = 3.187$, $p = 0.022$). As shown in Fig 4.3 (b) early responses increased to the greatest extent at 0.25mg/kg, with the highest 1.0mg/kg dose, in contrast, displaying a slight decrease in comparison to saline. Post hoc tests however revealed no significant differences between treatment conditions.

Inappropriate magazine entries during the stimulus presentation increased significantly following nicotine treatment during Go trials ($X^2 = 15.159$, $df = 4$, $p = 0.004$). A dose-related trend for nicotine to increase magazine entries was shown (Fig. 4.3 (c)). The effect reached significance at 0.5mg/kg and the highest 1.0mg/kg dose in comparison to saline (all $p < 0.05$). Furthermore, the highest dose enhanced this anticipatory measure significantly compared to 0.125mg/kg and 0.25mg/kg treatment conditions. Nicotine treatment had no significant effect on inappropriate magazine entries during No-go trials ($F(4,44) = 1.427$, N.S.) (see Fig. 4.3 (d)).

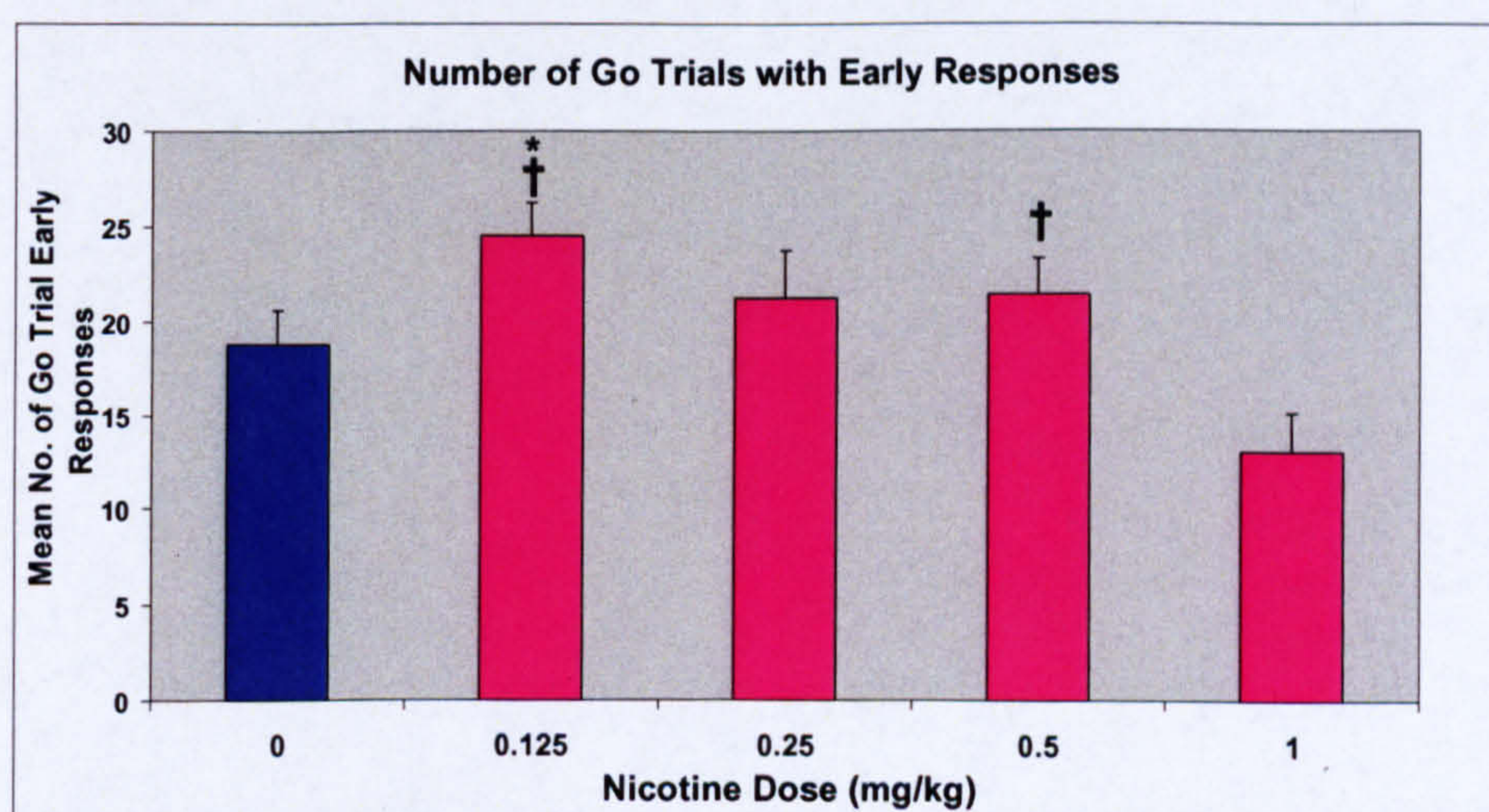


Fig. 4.3 (a)

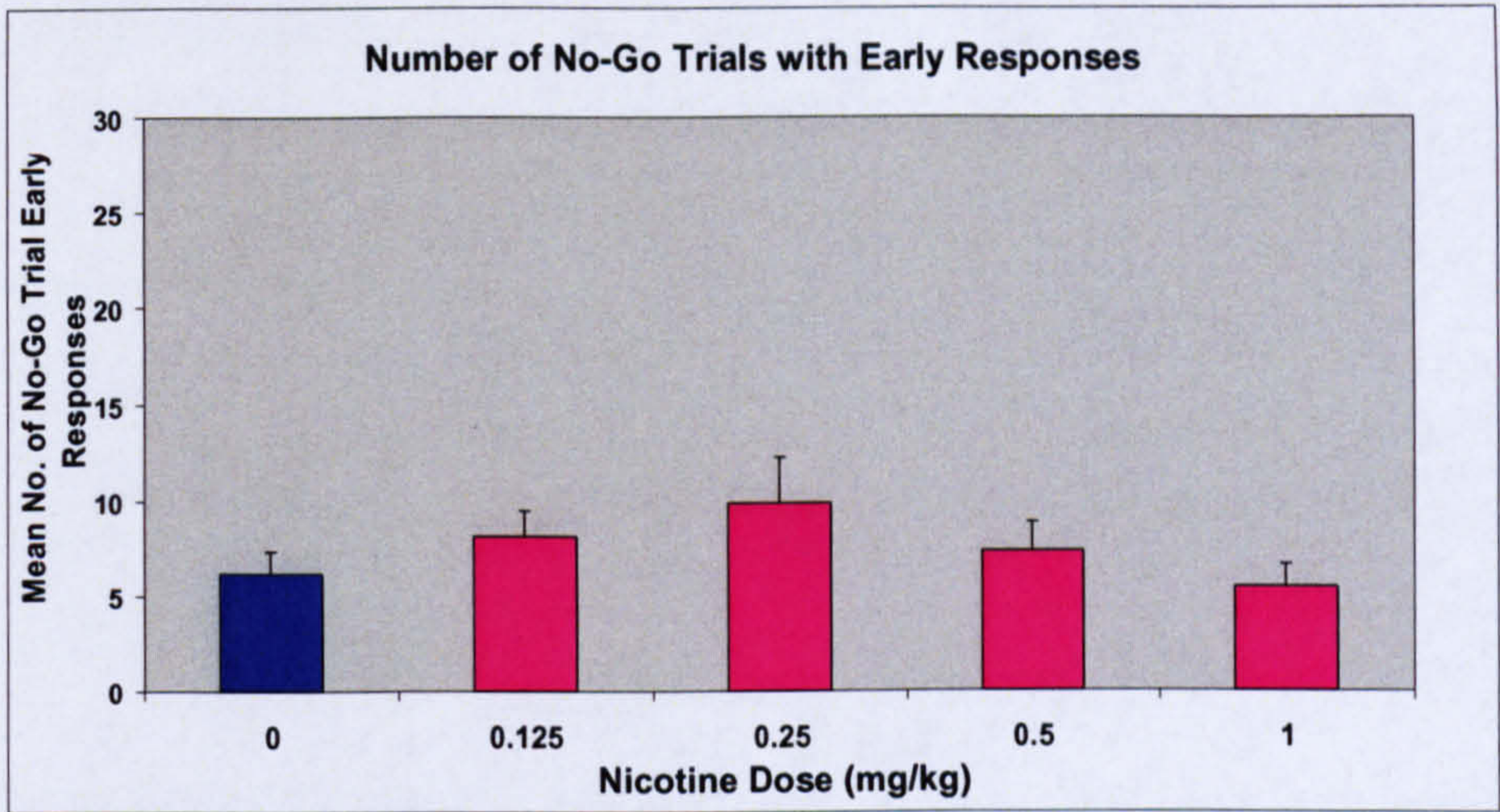


Fig. 4.3 (b)

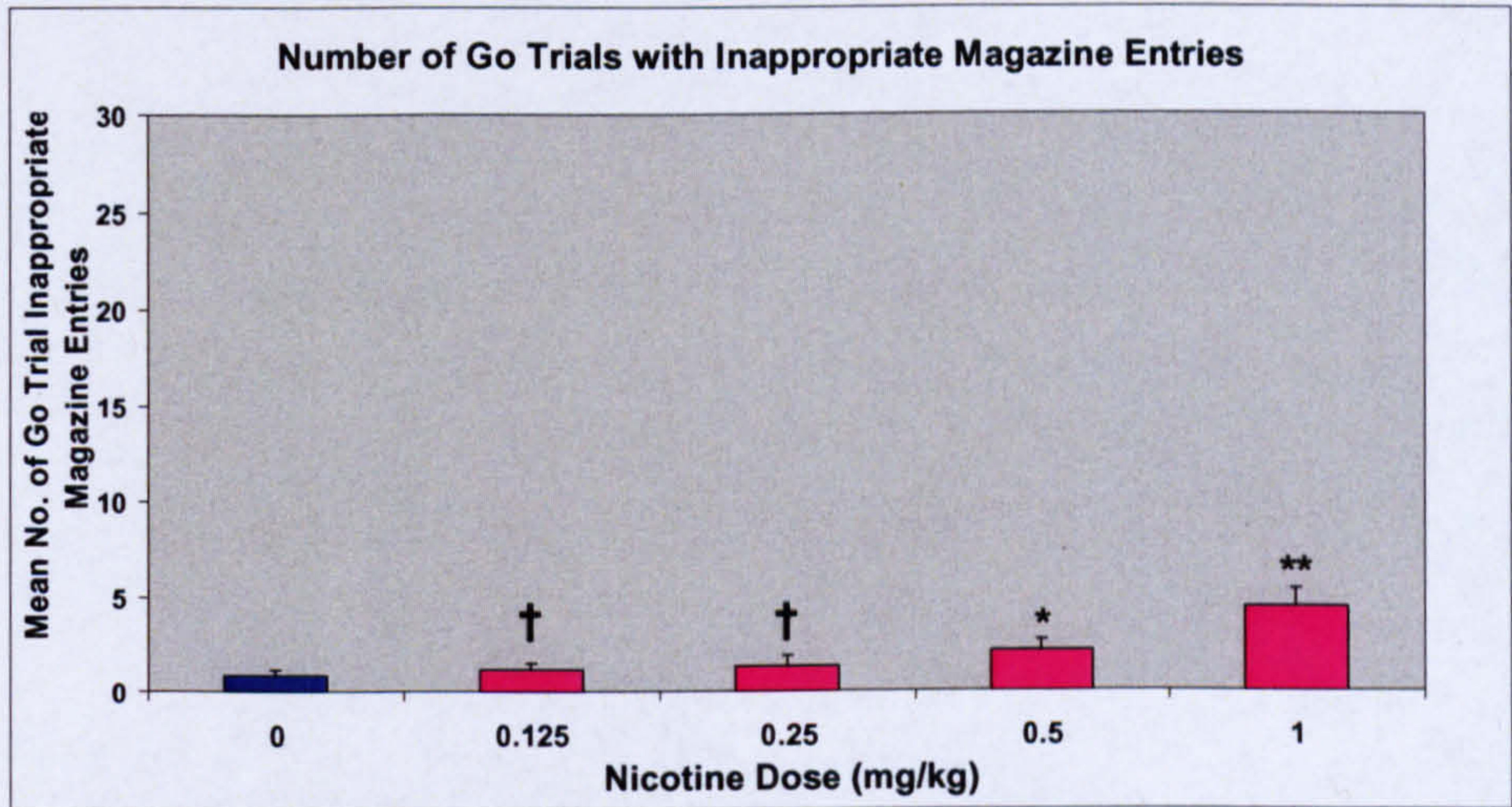


Fig 4.3 (c)

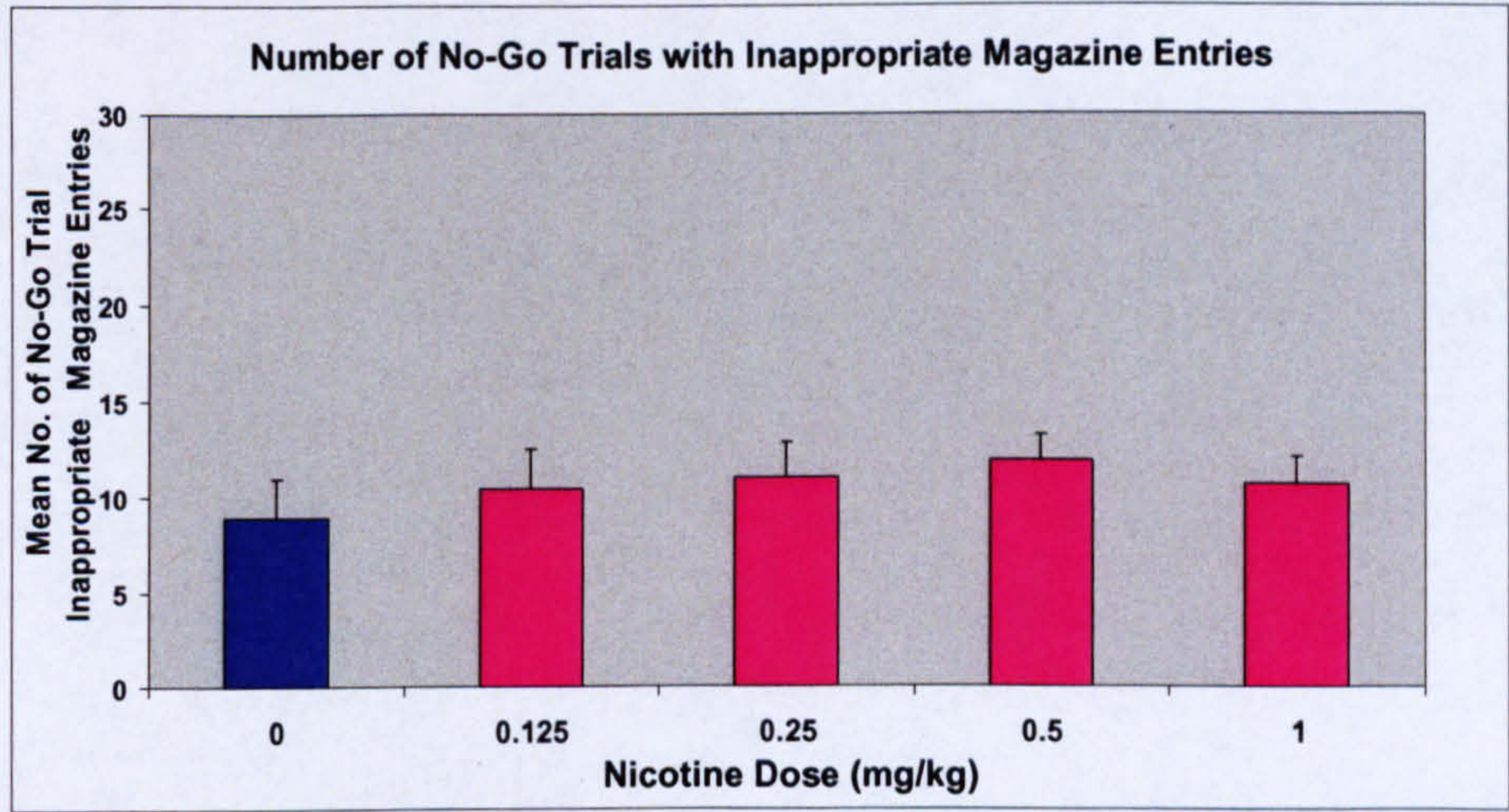


Fig. 4.3 (d)

Fig 4.3 (a-d): Anticipatory Responding: the effects of acute nicotine on early responses during Go trials (a) and No-go trials (b). Anticipatory Responding: the effects of acute nicotine on inappropriate magazine entries during Go trials (c) and No-go trials (d). Each bar represents the mean number of trials ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to saline control. †, p<0.05, ††, p<0.01, †††, p<0.001 (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

4.3.2.3. Speed of Responding

Table 4.1 summarises the effects of nicotine treatment on all measures of speed of responding. Analysis revealed a significant main effect of nicotine treatment on the latency to respond correctly during Go trials ($F(4,44) = 6.203$, $p < 0.001$). Further analysis of correct response latencies revealed that treatment with 1.0mg/kg nicotine resulted in significantly slower lever responding in comparison to saline control and 0.125mg/kg nicotine (all $p < 0.05$). However, the effect of treatment on incorrect response latencies during No-go trials failed to reach significance ($F(4,44) = 0.774$, N.S.).

Finally, nicotine had no significant effect on latencies to collect the reward during either Go or No-go trials ($F(1.831, 20.141) = 2.882$, $p = \text{N.S.}$; $X^2 = 9.067$, $df = 4$, $p = \text{N.S.}$, respectively).

Table 4.1: The effect of acute nicotine on speed of responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Nicotine Dose (mg/kg)				
	Saline (control)	0.125	0.25	0.5	1.0
Correct Response Latency	0.65 ± 0.08	$0.60 \pm 0.12^{\dagger\dagger}$	0.75 ± 0.09	0.82 ± 0.23	$1.45 \pm 0.31^*$
Incorrect Response latency	2.31 ± 0.31	2.04 ± 0.19	2.49 ± 0.25	2.65 ± 0.19	2.49 ± 0.44
Go magazine Latency	0.36 ± 0.07	0.31 ± 0.05	0.30 ± 0.02	0.32 ± 0.02	0.39 ± 0.04
No-go Magazine Latency	0.60 ± 0.11	0.49 ± 0.09	0.54 ± 0.08	0.54 ± 0.09	0.66 ± 0.06

Table 4.1: Each value represents the mean latency (seconds) \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

4.3.3. EXPERIMENT 2B: THE EFFECTS OF ACUTE ADMINISTRATION OF MECAMYLAMINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

4.3.3.1. Accuracy of Performance

Administration of MEC had no significant effect on overall accuracy of performance ($F(3,30) = 1.571$, N.S.) (Fig.4.4(a)). Furthermore, independent analysis of Go and No-go trials also demonstrated a lack of effect of the nicotinic antagonist on accuracy of performance in the task ($F(3,30) = 0.807$, N.S.; $F(3,30) = 1.463$, N.S. respectively) (see Fig. 4.4(b) and (c)).

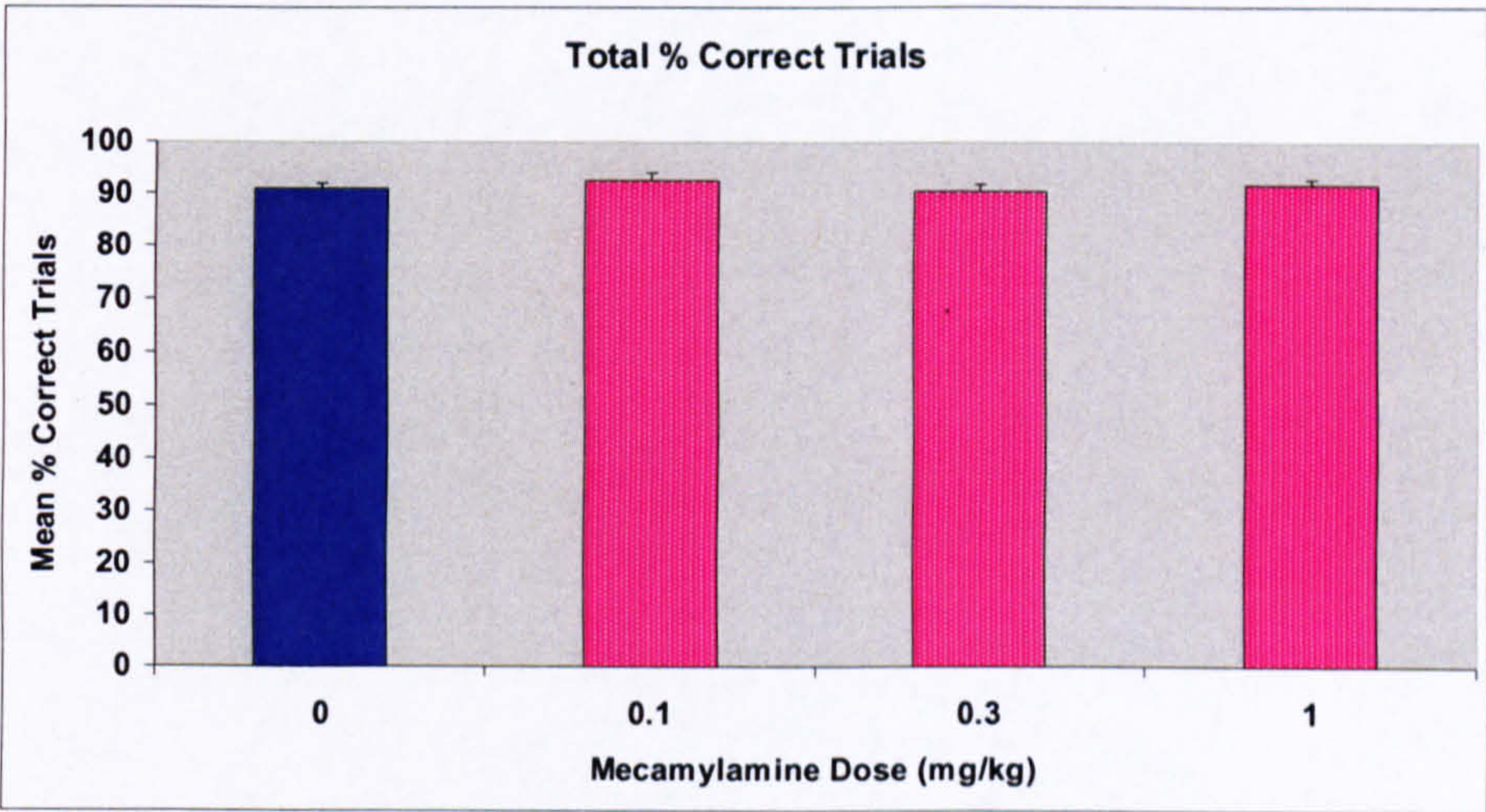


Fig. 4.4 (a)

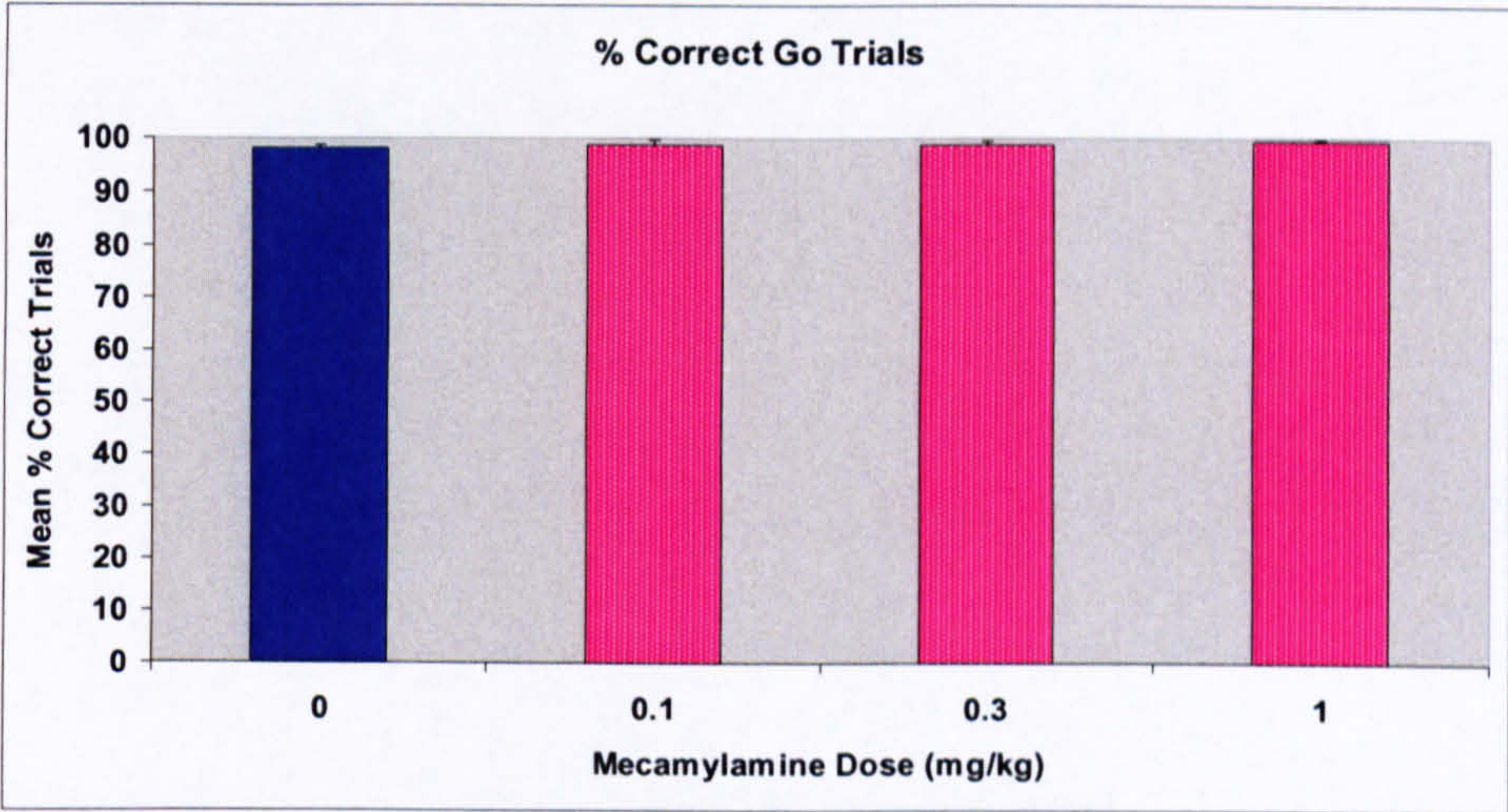


Fig.4.4 (b)

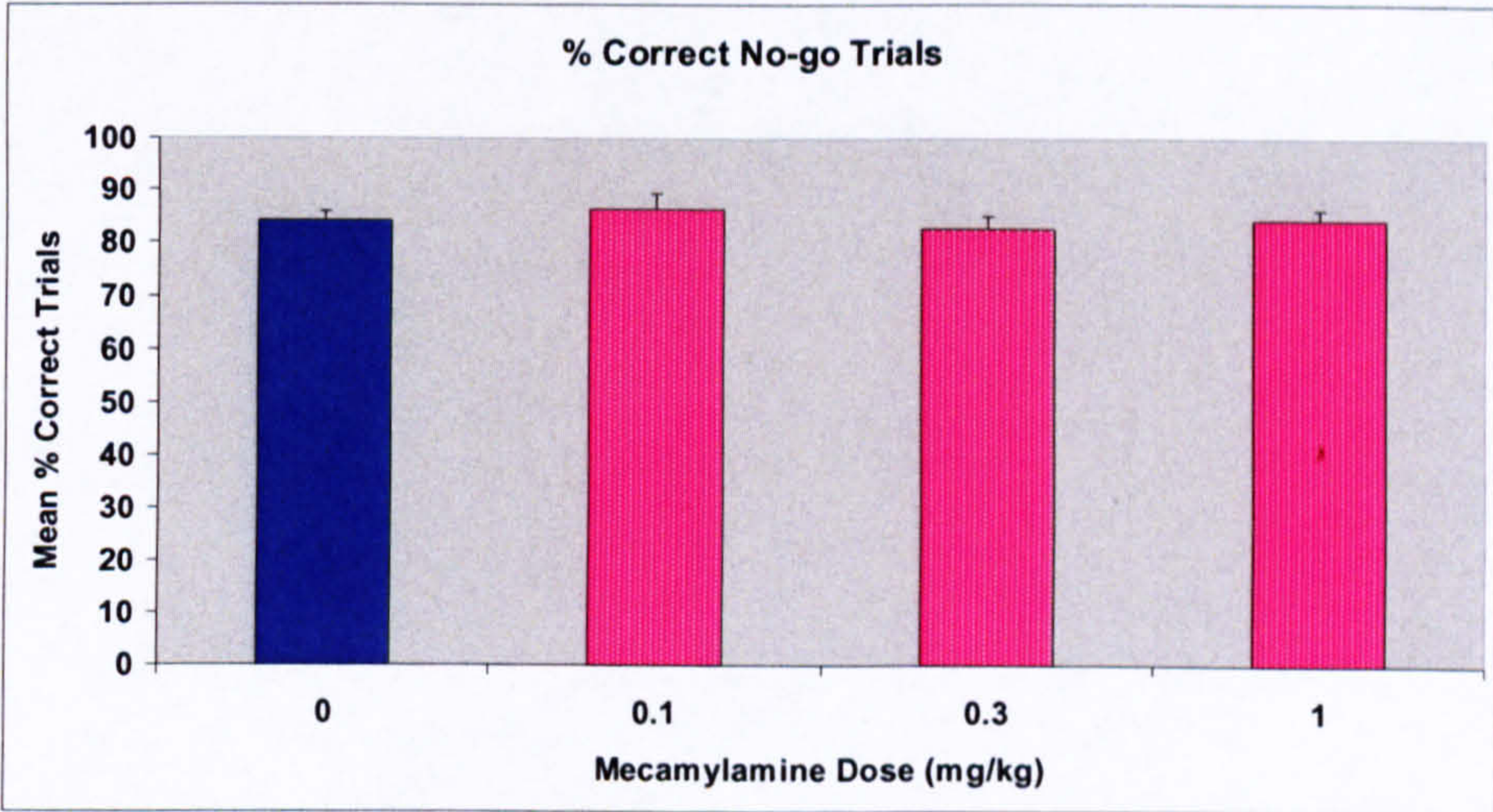


Fig 4.4 (c)

Fig 4.4 (a-c): Accuracy of Responding: the effects of acute mecamlamine administration on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each bar represents the mean percentage correct trials \pm SEM.

4.3.3.2. Anticipatory Responding

Table 4.2 summarises the effects of MEC on anticipatory responding. MEC failed to alter the frequency of early responding during Go trials ($F(3,30) = 0.117$, N.S.) and No-go trials ($F(3,30) = 0.898$, N.S.).

The frequency of magazine entries during Go trials also remained unchanged ($F(3,30) = 0.240$, N.S.). While a significant effect was observed on this behaviour during No-go trials ($F(3,30) = 3.882$, $p = 0.020$), post hoc tests however did not identify any significant differences between drug treatments.

Table 4.2: The effect of acute mecamlamine on anticipatory responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Mecamylamine Dose (mg/kg)			
	Saline (control)	0.1	0.3	1.0
Go trials with Early Responses	15.91 ± 2.66	17.09 ± 1.94	16.55 ± 1.74	16.73 ± 2.31
No-go trials with Early Responses	5.55 ± 1.57	4.82 ± 1.48	5.91 ± 1.34	6.45 ± 1.51
Go trials with Magazine Entries	0.82 ± 0.46	0.64 ± 0.24	1.09 ± 0.44	0.91 ± 0.31
No-Go trials with Magazine Entries	9.91 ± 2.32	6.18 ± 1.29	8.55 ± 2.16	7.73 ± 1.55

Table 4.2: Each value represents the mean frequency ± SEM.

4.3.3.3. Speed of Responding

The latency to correctly respond on the lever during Go trials did not differ following treatment ($F(3,30) = 0.741$, N.S.), (see Table 4.3). In contrast, analysis of incorrect response latencies during No-go trials displayed a significant main effect of treatment ($F(3,30) = 5.577$, $p = 0.004$). Post hoc comparisons revealed no significant differences between drug treatments and saline. The main effect instead reflected a significant decrease in latency to respond incorrectly at 0.1mg/kg in comparison to 0.3 and 1.0mg/kg doses of mecamlamine (all $p < 0.05$) (Table 4.3).

Finally no significant treatment effects were observed on magazine latencies during both Go and No-go trials ($F(1.180, 11.802) = 1.731$, N.S.; $F(1.910, 19.096) = 0.510$, N.S., respectively).

Table 4.3: The effect of acute mecamlamine on speed of responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Mecamylamine Dose (mg/kg)			
	Saline (control)	0.1	0.3	1.0
Correct Response Latency	0.83 ± 0.13	0.87 ± 0.12	0.75 ± 0.13	0.70 ± 0.09
Incorrect Response latency	2.02 ± 0.32	0.95 ± 0.17§†	2.17 ± 0.40	1.71 ± 0.28
Go magazine Latency	0.39 ± 0.09	0.32 ± 0.05	0.33 ± 0.06	0.35 ± 0.05
No-go Magazine Latency	0.70 ± 0.13	0.66 ± 0.10	0.68 ± 0.14	0.66 ± 0.10

Table 4.3: Each values represents the mean latency (seconds) ± SEM. §, $p < 0.05$, §§, $p < 0.01$, §§§, $p < 0.001$ (Bonferroni comparison) as compared to the 0.3mg/kg mecamlamine dose. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to the highest 1.0mg/kg mecamlamine dose.

4.3.4. EXPERIMENT 2C: THE EFFECTS OF CO-ADMINISTRATION OF MECAMYLAMINE AND NICOTINE ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

4.3.4.1. Accuracy of Performance

A main effect of treatment was observed ($F(4,40) = 18.761$, $p < 0.001$) (Fig.4.5(a)). Pairwise comparisons demonstrated that nicotine alone significantly reduced the number of correct trials in comparison to the saline/saline control ($p < 0.01$). Pre-treatment with MEC, across all doses, significantly antagonised the effects of nicotine on accuracy of overall performance, with co-administered doses of MEC/NIC failing to differ from the saline/saline control. Overall accuracy of performance on the task following all MEC/NIC combination treatments was significantly greater than performance following nicotine treatment alone (all $p < 0.01$).

Accuracy of performance on Go trials was not affected by nicotine or by its combination with the antagonist ($F(4,40) = 2.945$, N.S.) (see Fig. 4.5 (b)). Analysis of the accuracy of performance on No-go trials in contrast revealed a significant main effect of treatment ($F(4,40) = 20.512$, $p < 0.001$) (Fig. 4.5(c)). Nicotine, when administered alone significantly decreased the percentage of correct No-go trials relative to saline/saline control ($p < 0.01$). Post hoc analysis revealed that pre-treatment with MEC across all doses blocked the effects of nicotine, as demonstrated by the lack of difference in comparison to the saline/saline dose. Furthermore, nicotine treatment alone differed significantly from all MEC/NIC combination treatments (all $p < 0.01$).

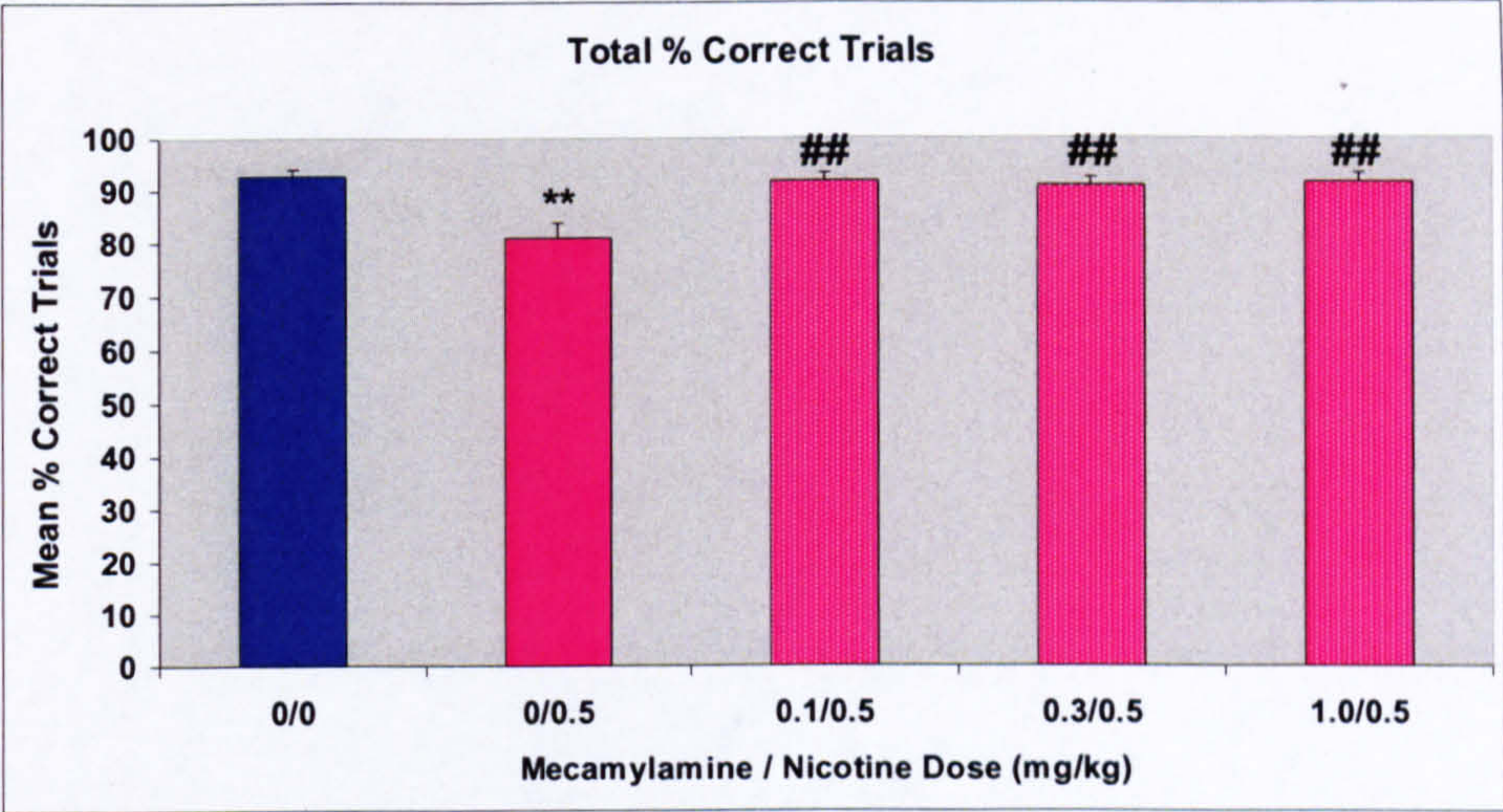


Fig 4.5 (a)

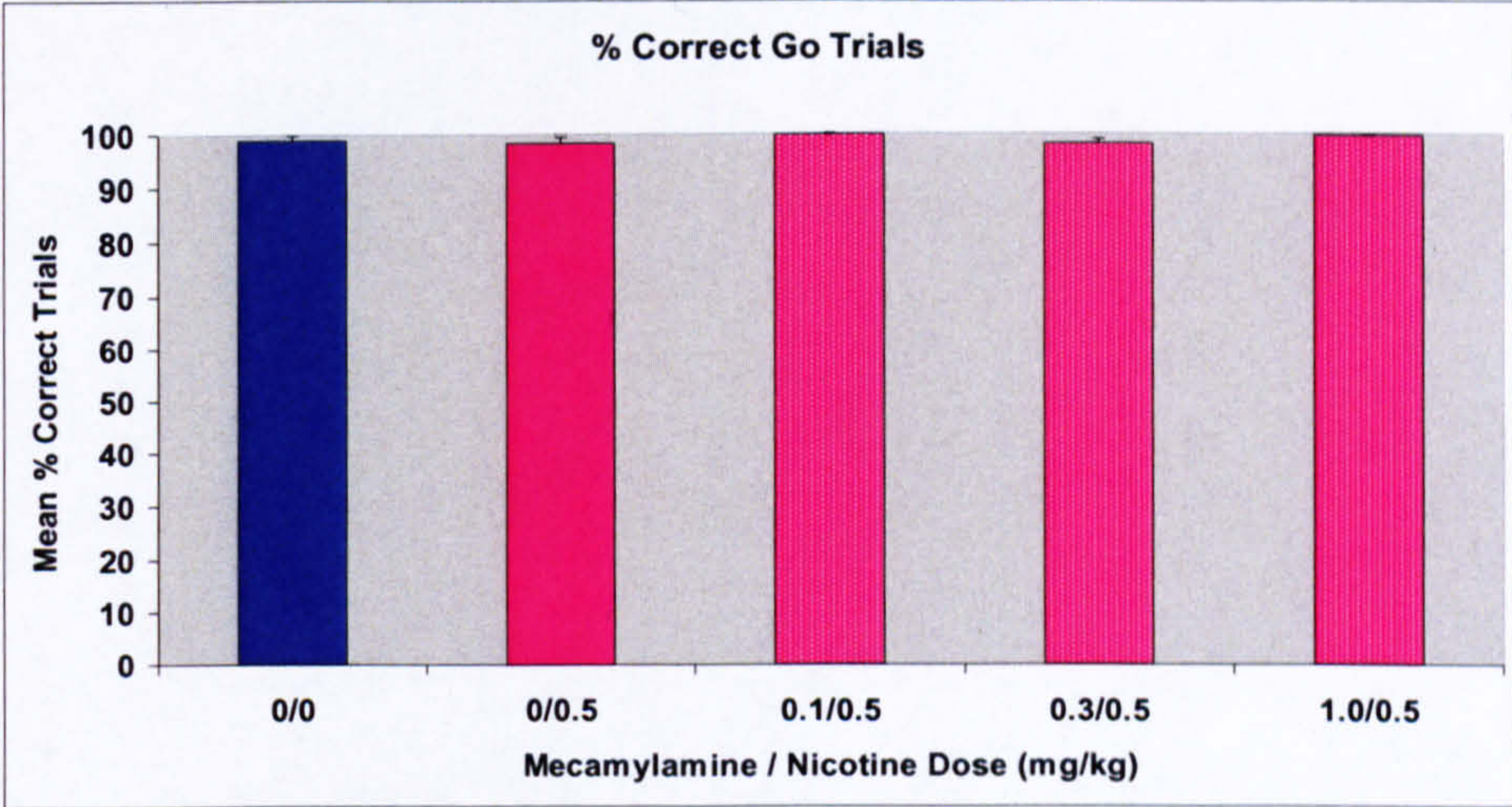


Fig 4.5 (b)

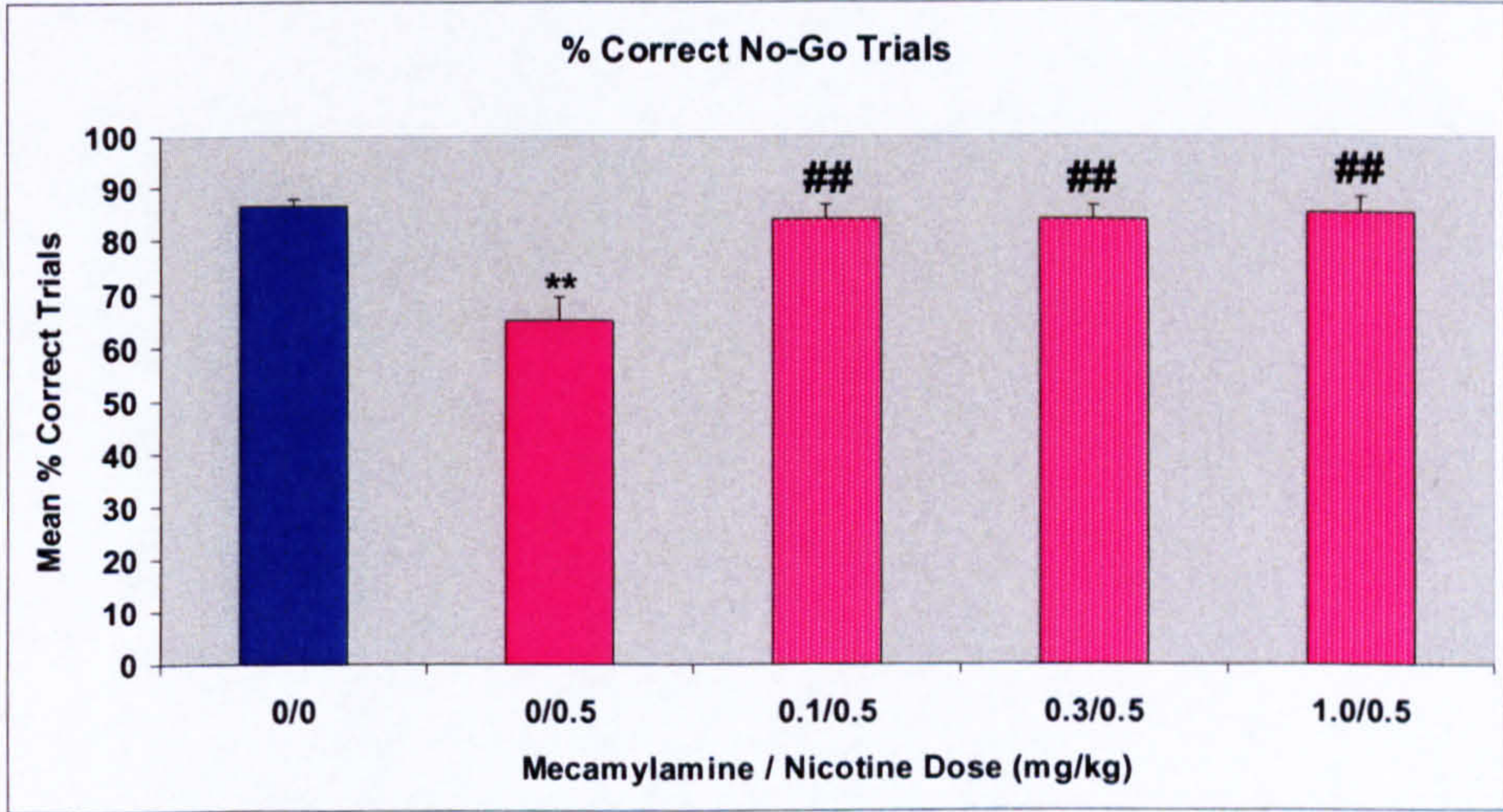


Fig 4.5 (c)

Fig 4.5 (a-c): Accuracy of Responding: the effects of co-administration of nicotine and mecamylamine on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each bar represents the mean percentage correct trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline/saline control. #, $p < 0.05$, ##, $p < 0.01$, ###, $p < 0.001$ (Bonferroni comparison) as compared to nicotine treatment alone (0/0.5mg/kg dose).

4.3.4.2. Anticipatory Responding

Despite the marginal increase in anticipatory responding following the administration of nicotine alone, analysis demonstrated no significant main effect of treatment for the frequency of early responses or inappropriate magazine entries during both Go and No-go trials (all $F(4,40) \leq 1.461$, N.S.) (see Table 4.4).

Table 4.4: The effect of co-administration of nicotine and mecamylamine on anticipatory responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Mecamylamine Dose (mg/kg) / Nicotine Dose (mg/kg)				
	Saline/ Saline (control)	Saline/ NIC _{0.5mg/kg} (control)	MEC _{0.1mg/kg} / NIC _{0.5mg/kg}	MEC _{0.3mg/kg} / NIC _{0.5mg/kg}	MEC _{1.0mg/kg} / NIC _{0.5mg/kg}
Go trials with Early Responses	15.0 ± 1.96	15.46 ± 1.69	17.82 ± 1.99	18.18 ± 2.20	14.18 ± 1.68
No-go trials with Early Responses	5.45 ± 1.36	6.73 ± 1.20	5.82 ± 0.98	5.09 ± 1.30	5.45 ± 1.43
Go trials with Magazine Entries	0.73 ± 0.24	1.27 ± 0.71	0.91 ± 0.39	1.18 ± 0.55	1.45 ± 0.37
No-Go trials with Magazine Entries	8.18 ± 1.87	10.09 ± 1.44	8.91 ± 1.47	8.73 ± 1.56	8.27 ± 1.59

Table 4.4: Each value represents the mean frequency ± SEM.

4.3.4.3. Speed of Responding

In contrast to Experiment 2A, nicotine significantly increased the latency to incorrectly respond on the lever during No-go trials ($F(4,40) = 5.694$, $p = 0.001$). This effect, which differed significantly from saline ($p < 0.05$), was blocked by all pre-treatment doses of MEC (Table 4.5). Analysis of the speed at which animals responded on the lever during Go trials revealed no significant main effects of treatment ($F(4,40) = 0.320$, N.S.).

No significant effects were observed across drug treatments on the latencies to collect the reward following either a correct Go or No-go trial ($F(1.765, 17.647) = 2.300$, N.S.; $F(2.196, 21.917) = 1.781$, N.S., respectively). Table 4.5 summarises the effects of combination treatment on speed of responding.

Table 4.5: The effect of co-administration of nicotine and mecamylmaine on speed of responding in the symmetrically reinforced go/no-go task

Behavioural Measure	Mecamylamine Dose (mg/kg) / Nicotine Dose (mg/kg)				
	Saline/ Saline (control)	Saline/ NIC _{0.5mg/kg} (control)	MEC _{0.1mg/kg} / NIC _{0.5mg/kg}	MEC _{0.3mg/kg} / NIC _{0.5mg/kg}	MEC _{1.0mg/kg} / NIC _{0.5mg/kg}
Correct Response Latency	0.82 ± 0.10	0.82 ± 0.17	0.71 ± 0.11	0.78 ± 0.13	0.77 ± 0.11
Incorrect Response latency	1.49 ± 0.10	3.19 ± 0.17*	1.78 ± 0.31	1.64 ± 0.34	1.53 ± 0.33
Go magazine Latency	0.38 ± 0.11	0.32 ± 0.02	0.30 ± 0.04	0.33 ± 0.08	0.30 ± 0.03
No-go Magazine Latency	0.76 ± 0.09	0.61 ± 0.01	0.64 ± 0.11	0.74 ± 0.13	0.76 ± 0.20

Table 4.5: Each values represents the mean latency (seconds) ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to saline control. #, p<0.05, ##, p<0.01, ###, p<0.001 (Bonferroni comparison) as compared to nicotine treatment alone (0/0.5mg/kg dose).

4.4. DISCUSSION

The differences in levels of inhibitory control between smokers and non-smokers could be due to the direct effects of nicotine on impulsivity (Spinella 2002; Yakir et al., 2007). To explore this theory Experiment 2(A-C), assessed, in drug naïve rodents, the effects of acute nicotine on behavioural disinhibition measured by the symmetrically reinforced go/no-go task. Furthermore, the role played by central nicotinic receptors in the mediation of nicotine's effects on behavioural disinhibition were also explored. The primary finding of the study was that nicotine induced disinhibition, an effect that was antagonised by doses of MEC that alone had no effects on performance in the task. Each of the findings of Experiment 2 A-C will be discussed in detail in the following section.

Experiment 2A demonstrated that in animals without a history of nicotine exposure acute nicotine treatment at doses of 0.125 and 0.25mg/kg were without effect on overall accuracy in the task. However, a significant increase in premature early responding during Go trials was observed at the 0.125mg/kg dose, indicating evidence of perhaps enhanced impulsivity. Unlike accuracy of responding this behaviour has no consequence in the task and therefore may be more sensitive to the heightened impulsivity produced by low doses of nicotine. In contrast, overall performance in the task was significantly reduced at the two highest doses of nicotine, 0.5mg/kg and 1.0mg/kg. Nicotine however produced differing behavioural profiles at each of

these doses. At the highest dose (1.0mg/kg) the reduction in accuracy can be attributed to the inability to actively respond during Go trials without affecting the ability to withhold responding during No-go trials. Although inappropriate magazine entries increased during Go trials following this dose, the significant slowing of correct lever responding and evidence of a reduction in early responding suggests an overall disruption in basic task performance at the highest 1.0mg/kg dose tested. Comparable deficits in general operant behaviour have been previously reported following this dose in the 5CSRTT procedure (e.g. Hoyle et al., 2006), and is most likely attributable to the often profound locomotor depression observed at higher acute doses of the drug (Morrison and Stephenson, 1972; Stolerman, Fink and Jarvic, 1973; Clarke and Kumar, 1983; Gordan, Meehan and Schechter, 1993).

Conversely, following treatment with the 0.5mg/kg nicotine the reduction in overall accuracy is clearly related to a significant decrease in the ability to withhold responding on No-go trials whilst Go trial performance remained unaffected. This pattern of accuracy of responding reflects a clear manifestation of nicotine induced behavioural disinhibition (Fletcher, 1993). The increased impulsiveness at this dose was additionally supported by the coincident enhancement of anticipatory responding as measured by the increased frequency of inappropriate magazine entries during Go trials. The lack of effect on response and magazine latencies following this dose furthermore suggests that the drug-induced changes in impulsive responding reflect a genuine deficit in behavioural inhibitory control, rather than being a secondary effect to changes in locomotor activity (Clarke and Kumar, 1983).

The ability of acute nicotine to augment disinhibited behaviour supports the theory that the heightened impulsivity in smokers (Spinella 2002; Yakir et al., 2006) may arise in part from the acute pharmacological effects of nicotine. These data are in agreement with previous preclinical research findings demonstrating enhanced premature responding in the 5CSRTT and DRL in rodents following acute nicotine treatment (e.g. Morrison, 1968; Bizot 1998; Mirza and Stolerman, 1998; Blondel et al., 2000; Popke et al., 2000a; 2000b; Stolerman et al., 2000; Hahn, et al., 2002; Bizarro, et al., 2004; Bruin et al., 2006). However, the results contradict other laboratory studies adopting the 5CSRTT that have failed to observe an increase in anticipatory responding (Mirza and Bright, 2001; Hahn et al., 2003; Day et al., 2007). The inconsistent findings of the acute effects of nicotine on impulsive responding in the 5CSRTT may be accounted for by the generally lower dose range used in these studies in comparison to the present research (0.001-0.4mg/kg). Furthermore, unlike the go/no-go task the 5CSRTT is primarily a model of sustained attention which may explain the differing effects of nicotine on behavioural parameters of impulsivity in each of these paradigms.

When comparing the findings to the human literature once again the present results appear to be

in opposition. Both a lack of effect and an *increase* in inhibitory control on the CPT and SST have been reported in human subjects following acute administration of nicotine (Levin et al., 1996b; Levin et al., 1998; Potter and Newhouse, 2004; Bekker et al., 2005a). The differing paradigms utilised to assess inhibitory control is most likely to account for the discrepancy in findings. Firstly in past research utilising the CPT the additional positive effects of nicotine found on sustained attention may have masked nicotine's induced effects on behavioural disinhibition in this particular paradigm (Levin et al., 1996b; Levin et al., 1998; Bekker et al., 2005a). Secondly, although the SST and go/no-go task are paradigms that both primarily assess inhibitory control, contrasting effects of drugs of abuse in each of these paradigms is not uncommon (e.g. Reynolds 2006b). The go/no-go task requires the ability to wait and withhold inappropriate responding while in contrast the SST requires the ability to stop an already initiated response and switch to an alternative behaviour. It may be that nicotine impairs only the former of these processes of inhibitory control, an argument further supported by that fact that evidence suggests that the two behaviours may be neurobiologically dissociated at the dopaminergic level (e.g. Liao and Cheng, 2000; Overtom et al., 2003; Eagle et al., 2007).

In contrast, across the dose range tested (0.1, 0.3, 1.0mg/kg) the nicotinic antagonist MEC in Experiment 2B displayed no effects on accuracy of responding in the task. Furthermore, anticipatory and speed of responding measures remained unchanged following its treatment in comparison to the saline control. The lack of effect of MEC on behavioural disinhibition extends previous research that reported no effects of the antagonist on impulsive responding in the 5CSRTT (Blondel et al., 2000). When co-administered with nicotine all 'silent' doses of MEC antagonised the nicotine induced loss of inhibitory control at the 0.5mg/kg dose (Experiment 2C). Consistent with previous findings in the 5CSRTT (e.g. Blondel et al., 2000), this result suggests that the acute effects of nicotine on behavioural disinhibition are mediated by centrally located nicotinic receptors. The lack of selectivity of MEC however does not enable conclusions to be made regarding the specific nAChR subtypes that may be involved in this particular behavioural effect of nicotine. There is some evidence from previous research to suggest that $\alpha 4\beta 2$ and $\alpha 7$ receptors may play a fundamental role. For example, several studies have reported that administration of the competitive antagonist DH β E, which has relative selectivity for the $\alpha 4\beta 2$ and $\alpha 4\beta 4$ receptors (Harvey and Luetje, 1996; Chavez-Nriega et al., 1997), blocks the effects of nicotine on anticipatory responding in 5CSRTT (Blondel et al., 2000; Grottick and Higgins, 2000). Combined with the finding that stimulation of $\alpha 4\beta 2$ receptor by the agonist SIB 1765F is capable of increasing premature responding in the task (Grottick and Higgins, 2000), indicates strong evidence of the potential role of the $\alpha 4\beta 2$ receptor in the modulation of nicotine induced disinhibition in the 5CSRTT. More recently, increased interest has also been focused upon the participation of the homomeric $\alpha 7$ receptor. In $\alpha 7$ nicotinic knockout mice anticipatory responding was found to be increased significantly relative to wild

type mice in models comparable to the 5CSRTT (Keller et al., 2005; Hoyle et al., 2006). At present, however, it is unknown whether the nicotinic receptors that govern anticipatory responding in the 5CSRTT also influence disinhibited behaviour in the go/no-go task. This question could be addressed by future studies examining the role of both the $\alpha 4\beta 2$ and $\alpha 7$ receptors in nicotine-induced impulsivity in the go/no-go paradigm.

When considering the possible neural systems that may be mediating the nicotine induced effects on impulsivity, the mesocorticolimbic DA system is a likely candidate. When nicotine is administered acutely, as with other psychostimulants, striatal dopaminergic neurotransmission is enhanced (Di Chiara and Imperato, 1988). More specifically, stimulation of nAChRs receptors located within the VTA led to a dose-dependent increase in extracellular levels of DA in the shell of the NAc, believed to play a crucial role in nicotine's positive reinforcing properties (Benwell and Balfour, 1992; Nisell et al., 1994; Di Chiara and Imperato, 1988; Cadoni and Di Chiara, 2000). Interestingly both the $\beta 2$ (Picciotto et al., 1998; Sharples et al., 2000;) and $\alpha 7$ (Schilstrom et al., 1998) receptor subtypes have been implicated in acute nicotine induced DA release in the NAc and may be involved in the mediation of nicotine's effects on inhibitory control (Blondel et al., 2000; Grottick and Higgins, 2000; Hoyle et al., 2006;). This suggests that nicotine-induced disinhibition may depend heavily on the stimulation of DA activity within the NAc via the $\alpha 7$, $\beta 2$ nicotinic receptors. Lending support for this theory is the finding that blockade of the DA D1 and D2 receptors significantly attenuated nicotine, cocaine and amphetamine induced impulsive responding in the 5CSRTT (van Gaalen et al., 2006), indicating a crucial role of dopamine receptor activation in psychostimulant induced disinhibition. Implicating more directly the role DA activation in the NAc, a recent study by Pattij et al (2007) demonstrated that enhanced impulsive responding following amphetamine in the 5CSRTT was blocked by administration of the D2 antagonist, eticlopride in the NAc.

It is important to highlight that nicotine via presynaptic and postsynaptic nAChRs within the CNS influences the release of additional neurotransmitters that may have also played a role in the mediation of nicotine induced disinhibition in the present research. Administration of nicotine has been shown to lead to the release of acetylcholine, noradrenaline, 5-HT, glutamate and GABA (Wonnacott et al., 1990; Wonnacott, 1997; Li et al., 1998; Jones et al., 1999). Of particular interest is 5-HT, which has been found to be elevated in the frontal cortex regions following systemic administration of nicotine (Ribeiro et al., 1993; Nisell et al., 1996). As both the frontal cortex and 5-HT have been implicated in the neuronal processes related to inhibitory control (e.g. Harrison et al., 1999; Picton et al., 2007), the nicotine induced disruption of 5-HT and DA within the frontal cortex is therefore likely to have contributed to the deficits in inhibitory control observed following acute exposure. Since the neurobiological events downstream from nicotinic receptors were not explored in the present research, the proposed

mechanisms that may underlie nicotine induced disinhibition remain highly speculative and should be a focus of future research.

The finding that acute nicotine reduced the ability to withhold inappropriate responding during No-go trials suggests that the stimulant increased disinhibition. The heightened impulsivity is consistent with the observed dysfunctional levels of inhibitory control observed in heavy smokers (Spinella, 2002; Yakir et al., 2007), and suggests that the association between impulsivity and nicotine dependence may, in part, be explained by nicotine induced effects on inhibitory control. However, several alternative explanations of the present findings need to be considered. Firstly nicotine's effects on impulsive responding may have been governed through the psychostimulants anorectic effects (McNair and Bryson, 1983; Grunberg et al., 1986; Levin et al., 1987; Blaha et al., 1998; Miyata et al., 1999; Zhang et al., 2001). This however is unlikely to be the case, the effects of reduction in motivation for reward through pre-feeding in Experiment 1C displayed no effects on performance in the task. Furthermore, no effect on latency to collect reward was observed following the acute administration of nicotine, providing strong evidence that findings were not indicative of changes in primary motivation.

Secondly, the poorer performance in the task following nicotine treatment could reflect drug induced cognitive deficits that may impact upon accuracy during No-go trials. For example, impairment in conditional discrimination and breakdown in previously learned stimulus-reward contingencies could lead to an active responding bias. It could also be argued that both attentional and working memory processes are required for accurate performance in the go/no-go task, processes that may become disrupted following drug treatment. During the task animals are required to continually monitor the environment in order to be alerted when to, and when not to, respond and to recall the required responses associated with the discriminative stimulus. Previous research has however indicated that nicotine can *improve* conditional discrimination, attention and working memory, positive effects that have been established in both humans and animals (e.g. Terry et al., 1996; Levin et al., 1998; Mirza and Stolerman, 1998; Decamp and Schneider, 2006; Rusted and Trawley, 2006; Spinelli et al., 2006; Day et al., 2007). Taken together, these findings suggest that observed loss of inhibitory control in the task is unlikely to be a consequence of a disruption in cognitive processes.

Thirdly, based on evidence suggesting that nicotine increases intolerance to delayed gratification (Dallery and Locey, 2005); the poorer performance in the task could reflect an inability to tolerate the delayed delivery of reward during No-go trials rather than disinhibited behaviour. As previously argued by Harrison et al. (1999), if reward in the go/no-go task decreased in value with delay then it would be expected that a reduction in latency to incorrectly respond during No-go trials would be demonstrated following nicotine treatment. No such effect

was observed following the acute administration of nicotine in the task. A related argument is that nicotine's known alterations in the perception of time (Pradhan and Dutta, 1970; Bizot, 1997; Carrasco et al., 1998) could have affected performance, in that the length of time required to withhold responding during No-go trials appeared longer. This explanation however cannot account for performance in this particular paradigm of impulsivity. Exteroceptive visual cues are presented to inform the animal when to and when not to lever respond during trials therefore eliminating the need for timing behaviour. This argument is further supported by the findings in Experiment 1D that demonstrated no effects on accuracy of responding in the paradigm during No-go trials when the duration of stimulus presentation was varied during the task, indicating that animals' responding was conditioned to the differing visual cues rather the duration of stimulus presentation.

It appears therefore that alternative explanations cannot easily account for the impaired performance during No-go trials, providing strong support that acute nicotine induces a genuine increase in impulsivity. Effectively establishing, in drug naïve animals, that acute nicotine can induce impairments in inhibitory control, suggests that the ability to terminate drug taking behaviour could begin to be compromised following initial nicotine exposure. The drug induced loss of control could thus lead to continued drug taking and possibly facilitate or increase the possibility of making the transition to dependence.

4.4.1. Conclusions

The main findings of Chapter 4 demonstrate that acute nicotine can induce disinhibition in the go/no-go task, an effect that appears to be mediated by central nicotinic receptors. These findings suggest that nicotine induced behavioural disinhibition may therefore be an important process underlying the early stages of addiction, whereby initial smoking may lead to a loss of control over future drug seeking and taking behaviour thus increasing the likelihood of the transition to dependence.

CHAPTER 5

The effects of chronic nicotine administration and nicotine withdrawal on behavioural disinhibition

5.1. INTRODUCTION

5.1.1. General Introduction

In order to better understand the role played by impulsivity in both the maintenance and relapse of drug abuse, it is important to assess the effects of chronic nicotine administration, nicotine withdrawal and the residual sensitivity to nicotine following a sustained period of abstinence on disinhibition. Although Experiment 2 clearly demonstrated that acute nicotine administration leads to a loss of inhibitory control, chronic drug regimes model the pattern of drug use in dependent smokers more precisely.

At present, the best evidence available to suggest that chronic drug use may increase disinhibition, is that heavier more dependent drug abusers display greater dysfunction in inhibitory control in comparison to less frequent, arguably non-dependent, drug users (Bolla et al., 2000; Spinella et al., 2002; Dinn et al., 2004; Yakir et al., 2007). Unfortunately, due to the cross sectional design of this research interpretation is hindered by the lack of information regarding pre-drug levels of impulsivity and therefore does not permit any valid conclusions to be made regarding the causality of the apparent dose-dependent-type relationship between disinhibition and dependence. Once again, limited research has been conducted investigating the effects of chronic drug administration on disinhibition. Research that has explored the effects of chronic cocaine has yielded mixed results. Paine and colleagues (2003) failed to demonstrate any changes in performance on a go/no-go task during 14 days of cocaine administration, whilst Jentsh et al. (2002) reported an increase in perseverative responding on an object discrimination task using a comparable drug regime. Research investigating the effects of nicotine has indicated that repeated, intermittent dosing of nicotine progressively increased anticipatory responding in a 5CSRTT, an attentional paradigm (Blondel et al., 1999).

Relapse into drug taking behaviour following abstinence commonly occurs in the first few weeks of abstinence, (Gossop et al., 1989; Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995). Therefore, assessing the effects of nicotine withdrawal on inhibitory control may indicate the importance of disinhibition as a possible psychological mechanism underlying drug relapse in smokers. To date, evidence has been provided of a

persistent dysfunction in inhibitory control in both abstinent cocaine users and alcoholics relative to control subjects at two weeks and three months following termination of drug consumption respectively (Gourdriaan et al., 2005; Verdejo-Garcia et al., 2007). In smoker's early nicotine withdrawal, 24hr abstinence, has been associated with an increase in anticipatory responding on the CPT (Hatsukami et al., 1989; Dawkins et al., 2007). However, following longer term nicotine abstinence, a minimum of six months, levels of disinhibition on the same task were found to be between that of non-smokers and smokers, but not significantly different from either group (Yakir et al., 2007). As discussed previously, the lack of longitudinal studies hinders the interpretation of these data. It is possible that early in drug withdrawal there is a loss of inhibitory control (Hatsukami et al., 1989; Dawkins et al., 2007), whereas later in drug abstinence this deficit recovers (Yakir et al., 2007). Conversely, the greater levels of inhibitory control over behaviour could have enabled these individuals to successfully abstain (Krishan-Sarin et al., 2007).

Although early stages of abstinence are associated with high rates of relapse, longer term vulnerability after 'quitting' is well documented (Stephens and Cottrell, 1972; Robinson and Berridge, 2000; 2003; Hughes et al., 2004; Piasecki, 2006; Hser, 2007). Current theories of drug addiction propose that impaired inhibitory control may increase the likelihood of drug relapse (Jentsh and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004). In support of this, long term drug taking leads to neuroadaptations including alterations of the serotonergic and dopaminergic systems in areas associated with inhibitory control, including the VTA and its associated projections (Kirch et al., 1987; Vezina et al., 1992; Ramussen and Czachura, 1995; Nisell, 1996; Balfour et al., 1998; Hildebrand et al., 1998; Harrison et al., 1999; Hildebrand, Panagis, Svensson and Nomikos, 1999; Olausson et al., 2001; de Wit et al., 2002; Christakou et al., 2004; Rahman et al., 2004; Picton et al., 2007). Thus, neuroadaptations resulting from chronic drug taking may render the individual vulnerable to relapse in which a loss of inhibitory control may be a key aspect. In relation to this, one of the strongest predictors of smoking relapse is the initial lapse of smoking a single cigarette during abstinence (Brandon et al., 1990; Nides et al., 1995; Shiffman et al., 1996). This in part may be mediated by neuroadaptations that leave the individual hypersensitive to the acute effects of nicotine on impulsivity leading to a loss of inhibitory control of future drug seeking and taking behaviour (relapse).

5.1.2. Objectives of Experiment 3 and 4

In an attempt to gain further understanding of the relationship between nicotine dependence and disinhibition the objectives of Experiments 3 and 4 were:

- i) To determine the effects of chronic nicotine exposure on disinhibition as assessed by the symmetrically reinforced go/no-go task (Experiment 3).

- ii) To assess changes in levels of behavioural disinhibition during early stages of withdrawal when smokers are particularly vulnerable to relapse (Experiment 3).
- iii) To assess the stability of changes in disinhibition during longer term abstinence (Experiment 3).
- iv) To determine whether chronic drug exposure leads to long term alterations in responsivity to the acute effects of nicotine on disinhibition (Experiment 4).

To achieve these objectives a longitudinal study was conducted in Experiment 3 during which the levels of inhibitory control of subjects was assessed in subjects prior to the initiation of drug treatment, during seven days of chronic nicotine exposure, and during initial and long term nicotine withdrawal. Following this, the same subjects were tested by a series of acute nicotine challenges in Experiment 4.

To verify that animals were experiencing nicotine withdrawal the nicotine abstinence syndrome was additionally assessed during Experiment 3 (Malin, et al., 1992). In animals nicotine deprivation consists of observable, somatic (physical) symptoms that can be induced by precipitated or spontaneous nicotine withdrawal (Malin et al., 1992; 1994; Hilderbrand et al., 1997; Watkins et al., 2000). These symptoms can be quantified to determine the intensity of withdrawal (Malin et al., 1992).

5.2. EXPERIMENT 3: ASSESSMENT OF THE EFFECTS OF CHRONIC NICOTINE ADMINISTRATION AND NICOTINE WITHDRAWAL ON BEHAVIOURAL DISINHIBITION

5.2.1. METHOD

5.2.1.1. Subjects

Subjects were 19 adult male Lister Hooded rats (Charles River, UK). On arrival animals were pair-housed and maintained under a 12 hour light/dark cycle (lights on at 0700h) at controlled environmental temperature of $21^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and relative humidity of $50\% \pm 10\%$. During experimentation food was restricted in order to maintain animals at 85% of their free feeding body weights. Water was freely available in home cages and feeding occurred at the end of each experimental day. At the start of testing, animals weighed approximately 330-360g. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1996.

5.2.1.2. Apparatus

Subjects performed the symmetrically reinforced go/no-go task in four operant chambers (dimensions 30.5 X 24.1 X 21cm Med Associates Inc., USA). During the observation of somatic withdrawal signs animals were placed in a glass observation tank (dimensions 40.5 X 37 X 11 cm). Behaviours were recorded by a video camera (JVC TK-128OE) positioned

horizontally in front of the observation chamber. The camera was relayed to a monitor (TM-1500PS) in an adjacent laboratory. For a more detailed description of all apparatus refer to Chapter 2 section 2.5.1 and 2.6.1.

5.2.1.3. Behavioural Testing

The behavioural procedure of the symmetrically reinforced go/no-go task, in addition to the pre-training required are outlined in detail within the general methodology of Chapter 3, section 3.2.1.3. The behavioural measures assessed within the task are also detailed in this section. Subjects were trained for approximately 8 weeks until criterion performance of 85% total correct trials was reached on two consecutive sessions. Once this level of accuracy had been achieved, sessions continued for a one week period to ensure stability of performance prior to testing.

5.2.1.4. Drugs

Nicotine was dissolved in 0.9% saline and the pH adjusted to approximately 6. Nicotine was chronically administered through s.c. implanted osmotic mini pumps (Model 2ML1; Alzet, Charles River, UK) and the concentration calculated so that animals received 3.16mg/kg/day (free base) for a seven-day duration. The drug solution was released at a rate of 10µl/hr (see also section 2.3.1.).

5.2.1.5. Design and Procedure

A mixed design was employed to assess the chronic effects of chronic nicotine on behavioural disinhibition, with treatment group (chronic saline ($n = 9$) or nicotine treatment ($n = 10$)) as the between subject factor and test day as the within subject factor. Animals were separated into treatment group prior to the implantation of osmotic pumps. Groups were balanced according to their baseline accuracy of performance within the task. Experimentation followed standard operant testing procedures, detailed in the general methodology of Chapter 2, section 2.5.2. Operant testing took place during the light phase of their LD cycle between 1000h and 1830h.

5.2.1.5.1. THE EFFECTS OF CHRONIC NICOTINE ADMINISTRATION AND NICOTINE WITHDRAWAL ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Once trained, animals were separated into two treatment groups and their baseline behaviour assessed in the operant task across a one week period, during which animals were tested once a day. Following the assessment of baseline behaviour osmotic pumps were surgically implanted. For a more detailed description of surgical procedures refer to the general thesis methodology of Chapter 2, section 2.4. Eighteen hours following the implantation of the osmotic pumps (or twelve hours following the initiation of drug release) animals were tested. During the eighteen hours post surgery animals recovered from surgery in their home cage prior to operant testing.

During the seven days of chronic nicotine administration, performance on the go/no-go task was assessed once each day.

On the seventh day of drug administration pumps were surgically removed, initiating spontaneous withdrawal from nicotine. Performance on the task during withdrawal began 12 hours following pump removal and continued for a three week period. In order to examine early effects of withdrawal on behavioural disinhibition, during the first two days of withdrawal animals conducted the task twice per day, at 12, 18, 36, and 42 hours post pump removal. Data from validation studies demonstrated that running animals twice per day on the task had little or no effect on performance (see Chapter 3). During the remaining withdrawal period animal's performance was then assessed once per day.

5.2.1.5.2. ASSESSMENT OF THE NICOTINE ABSTINENCE SYNDROME

The intensity of spontaneous withdrawal was assessed by observing the frequency of somatic signs. This was conducted using a nicotine abstinence scale developed by Malin et al., (1992). Briefly the somatic signs, including gasps, writhes, body shakes, head shakes, chews, teeth chattering, cheek tremors, paw tremors, genital grooming, foot licks, yawns, ptosis and scratches were recorded. A detailed description of these behaviours in addition to the procedure adopted for their assessment have been outlined previously in the general methodology of Chapter 2, section 2.6 and Table 2.1.

Prior to assessment all animals were habituated to both the test room and observation chamber on two consecutive days. Somatic signs were observed, the day before pump implantation (baseline assessment), the final day of chronic drug infusion, and during the first week following the termination of drug treatment. During withdrawal somatic signs were recorded for a ten minute period immediately following operant testing at 6, 12.5, 18.5, 24, 36.5, 42.5, 60.5, 84.5, 108.5, 132.5, 156.5 and 180.5 hours following pump removal, with the exception of observations at 6 and 24 hours when no operant testing took place. The time points of observational sessions were selected on the basis of the previously reported elevation in somatic signs during withdrawal (Malin et al., 1992; 1994; Hilderbrand et al., 1997; Epping-Jordan et al., 1998; Harrison et al., 2001).

5.2.1.6. Statistical Analysis

In the case of all statistical procedures data prior to analysis were assessed for normality and transformed where necessary (see also section 2.7). Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction. The homogeneity of variance of between subject variables was assessed by Levine's test. In all cases, α values of $p < 0.05$ were deemed statistically significant.

5.2.1.6.1. THE EFFECTS OF CHRONIC NICOTINE ADMINISTRATION AND NICOTINE WITHDRAWAL ON PERFORMANCE IN THE SYMMETRICALLY REINFORCED GO/NO-GO TASK

Comparison of performance on the task by each treatment group was analysed across each stage of treatment; including baseline, chronic drug administration, withdrawal week one, withdrawal week two and withdrawal week three. Data from seven operant sessions were examined for each stage with the exception of withdrawal week one where nine test sessions were performed. Magazine omissions (the failure to collect reward) rarely occurred across all treatment stages and therefore analysis of this behaviour was not conducted.

Assessment of differences in performance between treatment groups during baseline, involved a two-way mixed ANOVA conducted on all behavioural parameters (accuracy of performance, anticipatory responding and speed of responding), with treatment group (nicotine or saline) as the between subject factor and test session as the within subject factor. All significant main effects were analysed further by Bonferroni post hoc comparisons and interactions were followed where appropriate by simple effects analysis. A single one way ANOVA analysed the stability of behaviour across test days for each treatment group. Independent t-tests analysed differences between each group on behavioural measures at individual test sessions (Bonferroni correction of $p < 0.007$).

Within and across treatment groups, animals demonstrated variability in baseline performance across parameters within the task. To control for the possibility that pre-drug differences in behaviour may influence the dependent variable being measured, all data from chronic drug and withdrawal treatment stages were subjected to a two-way mixed analysis of covariance (ANCOVA) (Howell et al., 1992; Stevens 1992; Tabachnick and Fidell 2007). At each of the treatment stages behavioural parameters were assessed with the average baseline performance over seven days acting as the covariate within the model. This analysis enabled pre-drug differences in baseline behaviour to be controlled for, thus reducing error variance and allowing a more accurate assessment of the effects of chronic drug treatment and withdrawal on behaviour. For all ANCOVAs, treatment group represented the between subject factor, whilst test session the within subject factor. Significant main effects were explored further by post hoc Bonferroni tests. Significant treatment group x test day interactions were examined by simple effects analysis; where individual means compared were adjusted to control for the effects of the covariate. One-way repeated measures ANCOVAs examined by group the stability of behaviour across test days, whilst a univariate ANCOVA compared groups at each individual test day (drug week, Bonferroni correction of $p < 0.007$; withdrawal week 1, Bonferroni correction $p < 0.006$; withdrawal week 2-3, Bonferroni correction of $p < 0.007$).

Prior to all ANCOVA a further assumption assessed was that of homogeneity of regression. This was assessed via the examination of interactions of the covariate with both the within and between subject variables. If homogeneity of regression was found to be violated (i.e. variables interacted significantly with the covariate) then the use of ANCOVA was no longer appropriate. Though rare, in such instances data was expressed instead as a percentage change from baseline and analysed by a two-way mixed ANOVA (Tabchnick and Fidell, 2007).

5.2.1.6.2. ASSESSMENT OF THE NICOTINE ABSTINENCE SYNDROME

Total somatic signs were analysed using a two-way mixed factors ANOVA, with treatment group as the between subject factor and observational session as the within subject factor. Bonferroni post hoc comparisons were applied to the data following significant main effects, whilst one-way ANOVA and independent t-tests were used to examine further significant interactions. Individual categories of withdrawal symptomatology (see Table 2.1) were, in contrast, analysed non-parametrically due to violations of normality. Examination of somatic signs by treatment group across sessions were analysed by Freidman and Wilcoxon tests, while comparison between groups across sessions were performed by Mann Whitney procedural tests.

5.2.2. RESULTS

Figures 5.1 (a-c) illustrate the accuracy of performance within the task of each treatment group across all stages of study. All graphs represent actual mean values. The following sections in turn will investigate more closely differences in behaviour across treatment groups during each stage of study.

For all reported ANCOVA the effect of the covariate (average baseline performance) remained highly significant across assessment of all parameters within the task (all $F(1,16) \geq 6.908$, $p \leq 0.025$). This indicated that performance during both chronic drug treatment and withdrawal was significantly influenced by pre-drug differences in baseline behaviour, therefore supporting the importance of its control within analysis. With the exception of performance on Go trials, homogeneity of regression was satisfactory for all behavioural measures. A lack of interaction was found between the covariate and the independent variables of both treatment group (all stages: all $F(1,15) \geq 0.001$, N.S) and day (drug week: all $F(6,90) \geq 0.481$, N.S; withdrawal week one: all $F(8,120) \geq 0.123$, N.S ; withdrawal week two: all $F(6,90) \geq 0.225$, N.S; withdrawal week three: all $F(6,90) \geq 0.372$, N.S.). In the case of performance on Go trials, whilst the covariate was found not to interact with treatment group (all stages: all $F(1,16) \geq 0.006$, N.S), significant interactions were however consistently shown with the within subject factor of day (drug week: $F(6,90) = 4.000$, $p = 0.001$; withdrawal week one : $F(8,120) = 5.414$, $p < 0.001$; withdrawal week two : $F(6,90) = 3.734$, $p = 0.002$; withdrawal week three : $F(6,90) = 2.461$, $p = 0.020$). The violation of regression meant that this measure was therefore expressed as a

percentage change from baseline and analysed by a two-way mixed ANOVA. Unless otherwise stated, analysis of behavioural parameters during both chronic drug administration and stages of withdrawal displayed no significant main effects of test day on measures of accuracy of performance, anticipatory responding or speed of responding within the task.

5.2.2.1. BASELINE

5.2.2.1.1. Accuracy of Performance

Prior to treatment overall performance, and independent performance during both Go and No-go trials was stable with no main effects of test day found (all $F(6,102) \leq 2.047$, N.S.). Similar performance was observed across treatment groups as indicated by the absence of group main effects (all $F(1,17) \leq 0.320$, N.S.) and group x test day interactions found across all measures of accuracy during the seven day period (all $F(6,102) \leq 0.794$, N.S.) (see Fig. 5.1 (a-c)).

5.2.2.1.2. Anticipatory Responding

Analysis indicated no significant main effect of test day across all measures of anticipatory responding during baseline (all $F(6,102) \leq 1.162$, N.S.). Furthermore, no group differences were observed on the frequency of early responses or inappropriate magazine entries during both Go and No-go trials, with both non-significant between group main effects (all $F(1,17) \leq 1.749$, N.S.) or group x test day interactions found (all $F(6,102) \leq 0.792$, N.S.). See table 5.1 for summary of anticipatory responding across baseline week.

5.2.2.1.3. Speed of Responding

Table 5.2 summarises mean latency of speed of responding measures of both treatment groups across baseline week. Both correct and incorrect response latencies were stable during baseline with no main effect of test day observed (all $F(6,102) \leq 1.419$, N.S.). No differences were shown between treatment groups on the speed at which lever responses were made, either correctly during Go trials or incorrectly during No-go trials as shown by the non-significant main effects of group (all $F(1,17) \leq 3.507$, N.S.) and group x test day interactions (all $F(6,102) \leq 0.918$, N.S.).

Analysis of the speed with which animals collected reward demonstrated no significant main effect of test day for Go trial magazine latency ($F(6,102) = 1.187$, N.S.). Treatment groups furthermore did not differ on this speed of responding measure as shown by the lack of significant between group main effect ($F(1,17) = 0.302$, N.S.) and group x test day interaction ($F(6,102) = 0.918$, N.S.) found. Conversely, a significant main effect of test day was indicated for No-go magazine latency ($F(6,102) = 2.285$, $p = 0.041$), although post hoc analysis revealed no significant differences between baseline test days. Whilst a non-significant between group main effect was shown ($F(1,17) = 0.234$, N.S.), the group x test day interaction reached

significance ($F(6,102) = 4.059$, $p=0.001$). Further statistical analysis revealed a significant difference between groups on baseline day 3, with faster latencies performed by the group to be treated with nicotine ($t = 1.891$, $df = 17$, $p = 0.040$). Independent analysis of treatment group across baseline revealed a lack of significant main effect of test day for those animals that would be treated chronically with nicotine ($F(6,54) = 2.110$, N.S.). In contrast, the saline treatment group displayed a significant main effect of day ($F(6,48) = 3.993$, $p = 0.003$), although post hoc analysis failed to highlight significant differences in latency between baseline test sessions.

Table 5.1: Anticipatory responding in the symmetrically reinforced go/no-go task during baseline week

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Go trials with early responses	Nicotine	18.10 ± 1.75	18.20 ± 1.31	16.70 ± 1.37	16.20 ± 2.35	15.60 ± 1.67	17.50 ± 1.16	15.80 ± 2.06
	Saline	20.89 ± 2.71	19.33 ± 2.92	18.67 ± 2.73	20.00 ± 2.32	20.89 ± 2.29	18.67 ± 2.31	18.00 ± 2.27
No-Go trials with early responses	Nicotine	3.60 ± 0.86	4.20 ± 0.98	3.70 ± 0.87	3.50 ± 0.78	4.30 ± 0.88	5.00 ± 1.09	3.50 ± 0.79
	Saline	6.89 ± 1.78	6.56 ± 1.80	6.44 ± 2.05	6.00 ± 1.95	7.44 ± 2.28	7.89 ± 2.09	7.11 ± 2.08
Go trials with magazine entries	Nicotine	1.80 ± 0.53	1.50 ± 0.43	1.60 ± 0.40	1.40 ± 0.54	1.50 ± 0.52	1.20 ± 0.44	1.60 ± 0.54
	Saline	1.44 ± 0.78	1.33 ± 0.41	1.11 ± 0.26	1.56 ± 0.60	1.56 ± 0.80	1.56 ± 0.63	1.44 ± 0.63
No-go trials with magazine entries	Nicotine	13.33 ± 2.37	14.20 ± 2.65	14.50 ± 2.28	13.10 ± 1.91	14.50 ± 2.19	13.90 ± 2.23	16.90 ± 2.50
	Saline	10.89 ± 3.15	10.33 ± 2.95	9.67 ± 3.24	11.00 ± 3.29	12.33 ± 3.61	10.89 ± 4.03	9.56 ± 2.82

Table 5.1: Each value represents the mean frequency ± SEM.

Table 5.2: Speed of responding in the symmetrically reinforced go/no-go task during baseline week

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Correct response latency	Nicotine	1.09 ± 0.19	1.08 ± 0.20	1.08 ± 0.17	1.10 ± 0.24	1.10 ± 0.27	1.15 ± 0.24	1.25 ± 0.30
	Saline	0.71 ± 0.13	0.71 ± 0.15	0.80 ± 0.16	0.86 ± 0.15	0.84 ± 0.15	0.92 ± 0.16	0.83 ± 0.13
Incorrect response latency	Nicotine	2.37 ± 0.39	2.49 ± 0.30	2.06 ± 0.19	2.20 ± 0.34	1.71 ± 0.39	2.18 ± 0.33	1.62 ± 0.33
	Saline	1.65 ± 0.19	1.55 ± 0.35	1.60 ± 0.32	1.59 ± 0.22	1.74 ± 0.52	2.03 ± 0.43	1.23 ± 0.25
Go magazine latency	Nicotine	0.40 ± 0.03	0.38 ± 0.02	0.42 ± 0.05	0.37 ± 0.04	0.39 ± 0.04	0.40 ± 0.04	0.41 ± 0.04
	Saline	0.45 ± 0.13	0.42 ± 0.11	0.42 ± 0.11	0.39 ± 0.08	0.38 ± 0.09	0.38 ± 0.09	0.42 ± 0.10
No-go magazine latency	Nicotine	0.49 ± 0.04	0.46 ± 0.03	0.40* ± 0.02	0.43 ± 0.04	0.53 ± 0.05	0.47 ± 0.04	0.48 ± 0.03
	Saline	0.40 ± 0.04	0.45 ± 0.06	0.51 ± 0.04	0.57 ± 0.06	0.50 ± 0.04	0.48 ± 0.05	0.54 ± 0.04

Table 5.2: Each value represents the mean latency (seconds) ± SEM.*, p<0.05, (Bonferroni comparison) indicate significant differences between nicotine and saline treatment groups.

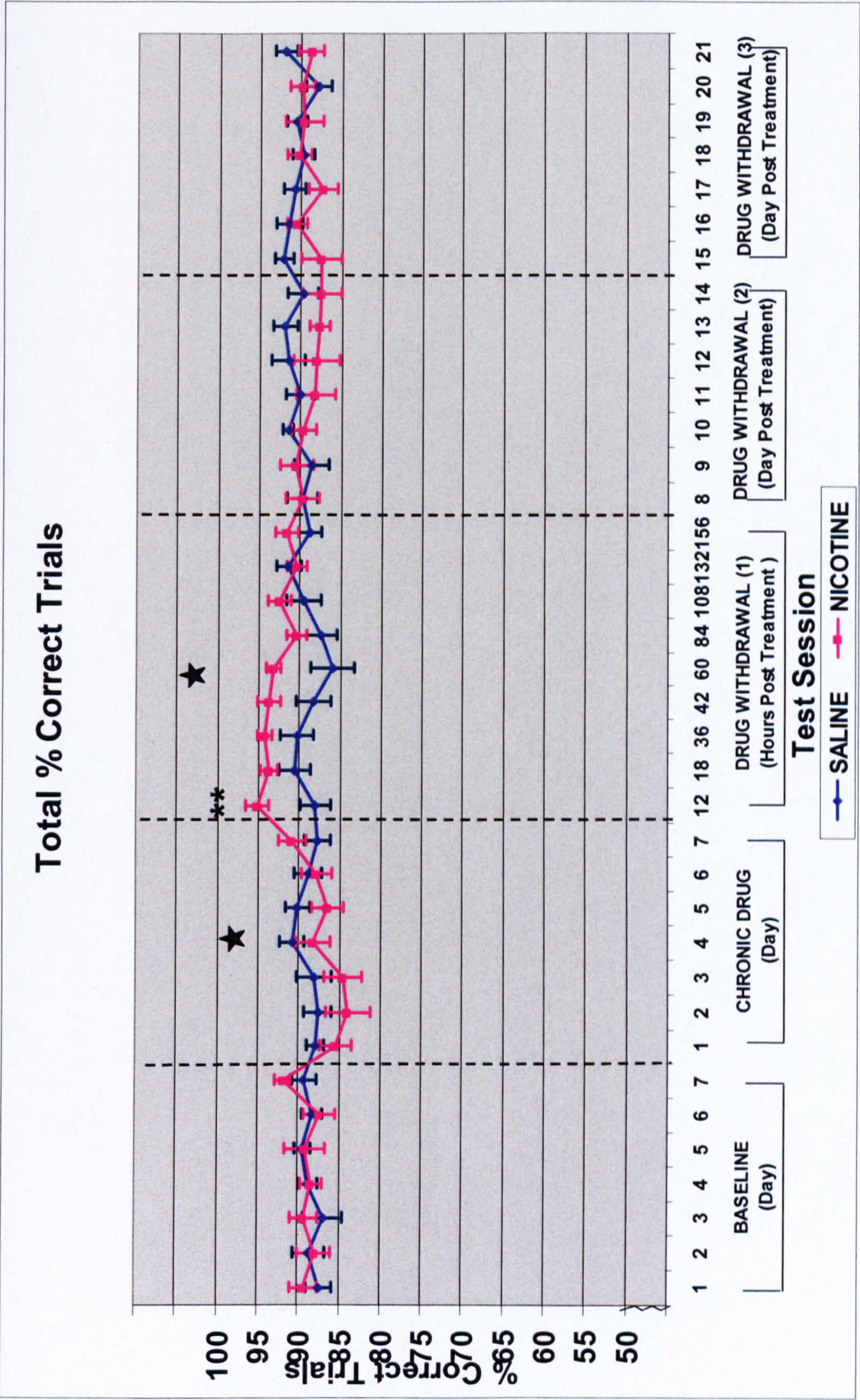


Fig. 5.1 (a)

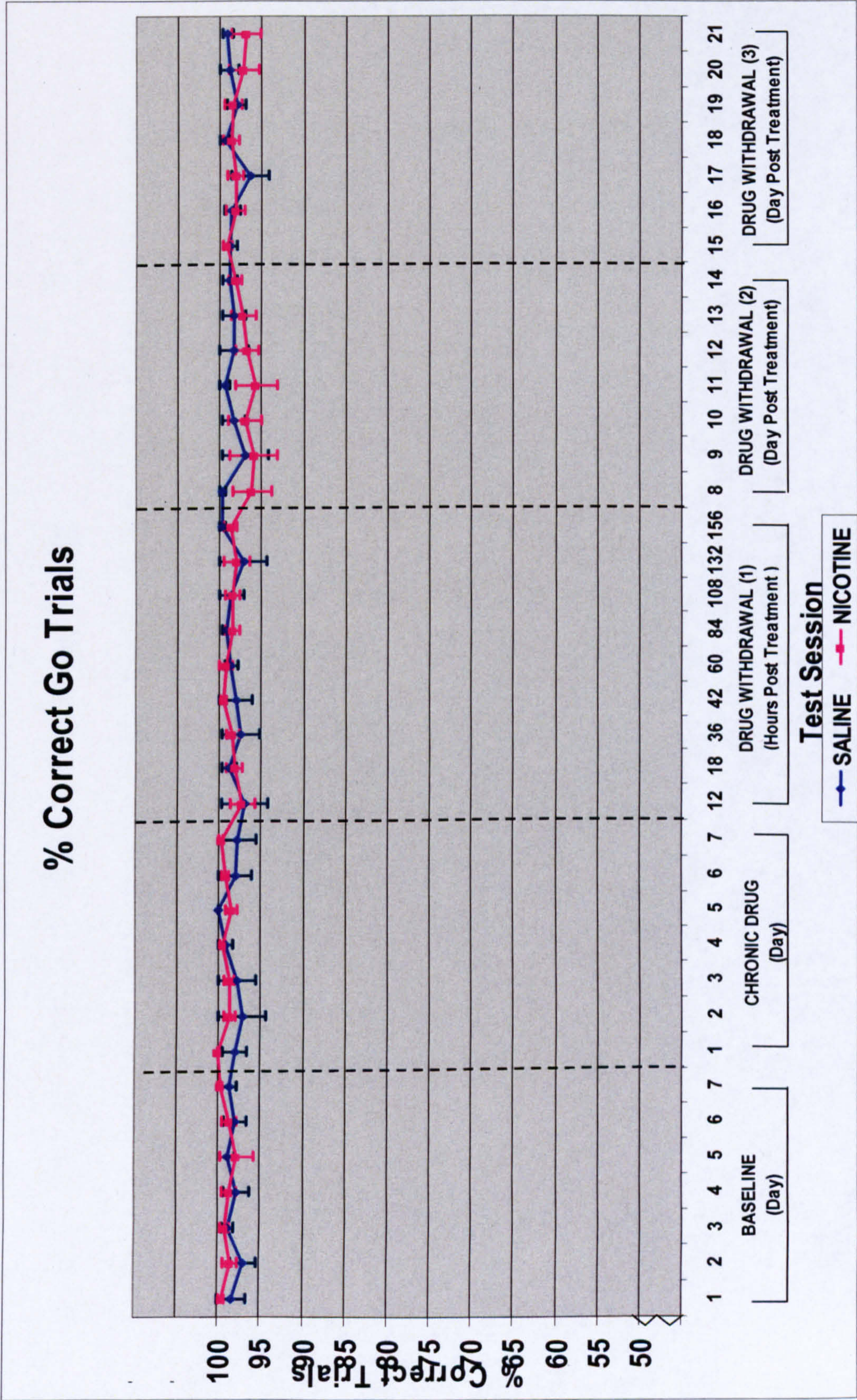


Fig. 5.1 (b)

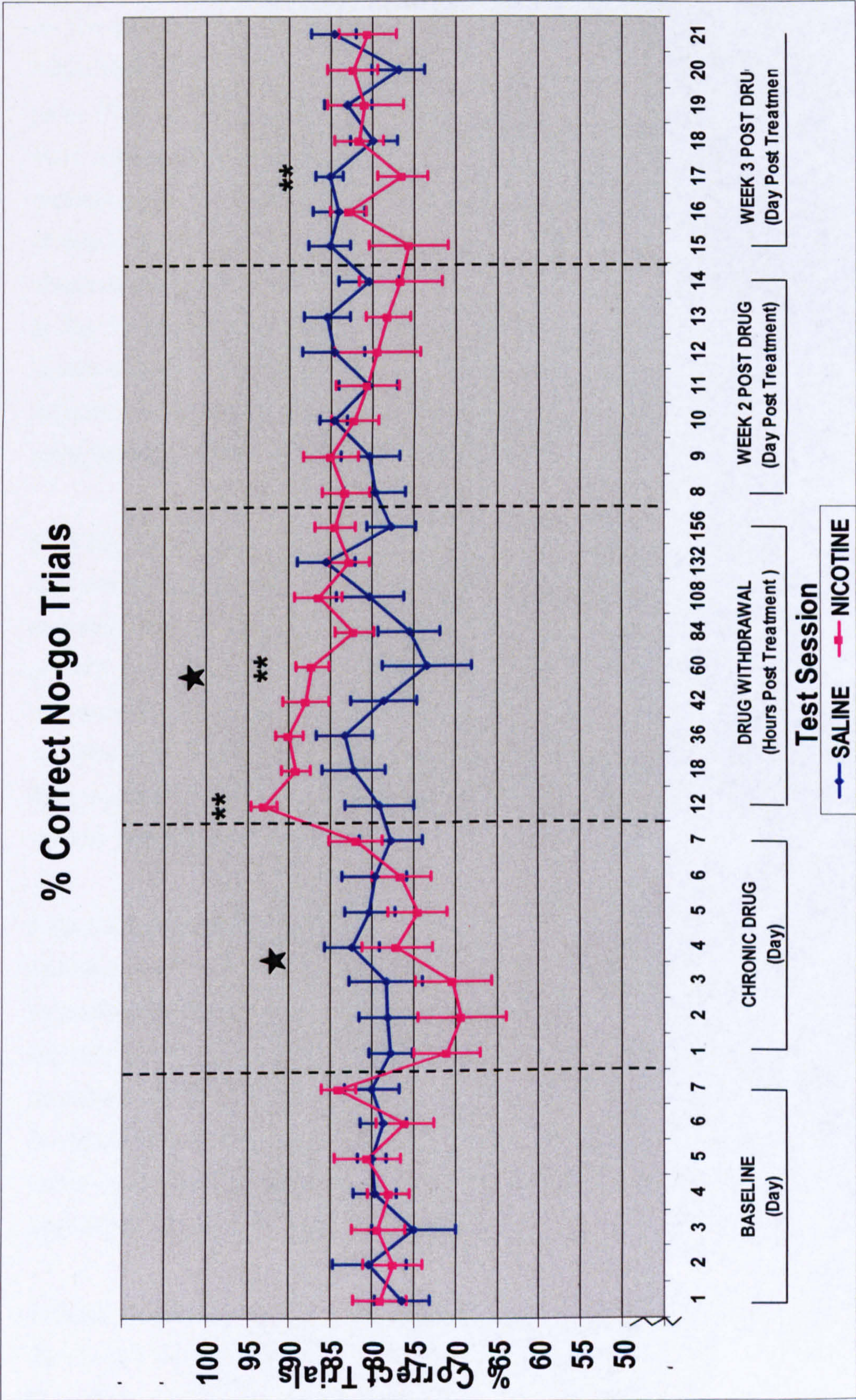


Fig. 5.1 (c)

Fig. 5.1 (a-c): Accuracy of Responding: the effects of chronic nicotine administration and withdrawal on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each point represents the mean percentage correct trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$ (Bonferroni comparison) indicate significant differences between nicotine and saline treatment groups. ★ $p < 0.05$ Main effect of group.

5.2.2.2. CHRONIC ADMINISTRATION OF NICOTINE

5.2.2.2.1. Accuracy of Performance

As illustrated in Fig. 5.1 (a), the initiation of chronic nicotine treatment led to a significant reduction in a overall accuracy of performance on the task in comparison to the saline treatment group ($F(1,16) = 6.076, p = 0.020$). Independent analysis of Go and No-go trials demonstrated that a greater inability to successfully withhold responding on No-go trials was displayed by the nicotine group as supported by the significant main effect of group on this measure of accuracy of responding ($F(1,16) = 5.961, p = 0.027$). In contrast no significant main effect of group was observed on performance during Go trials ($F(1,17) = 0.132, N.S.$) (see Figs. 5.1 (b-c)). As shown in Fig. 5.1 (a and c) despite the demonstration of greatest deficits in performance by nicotine treated animals at the early stages of treatment, no significant group x test day interactions were indicated across all measures of accuracy within the task (Total trials, $F(6,96) = 1.648, N.S.$; Go trials, $F(6,102) = 0.961$; No-go trials $F(6,96) = 0.737, N.S.$).

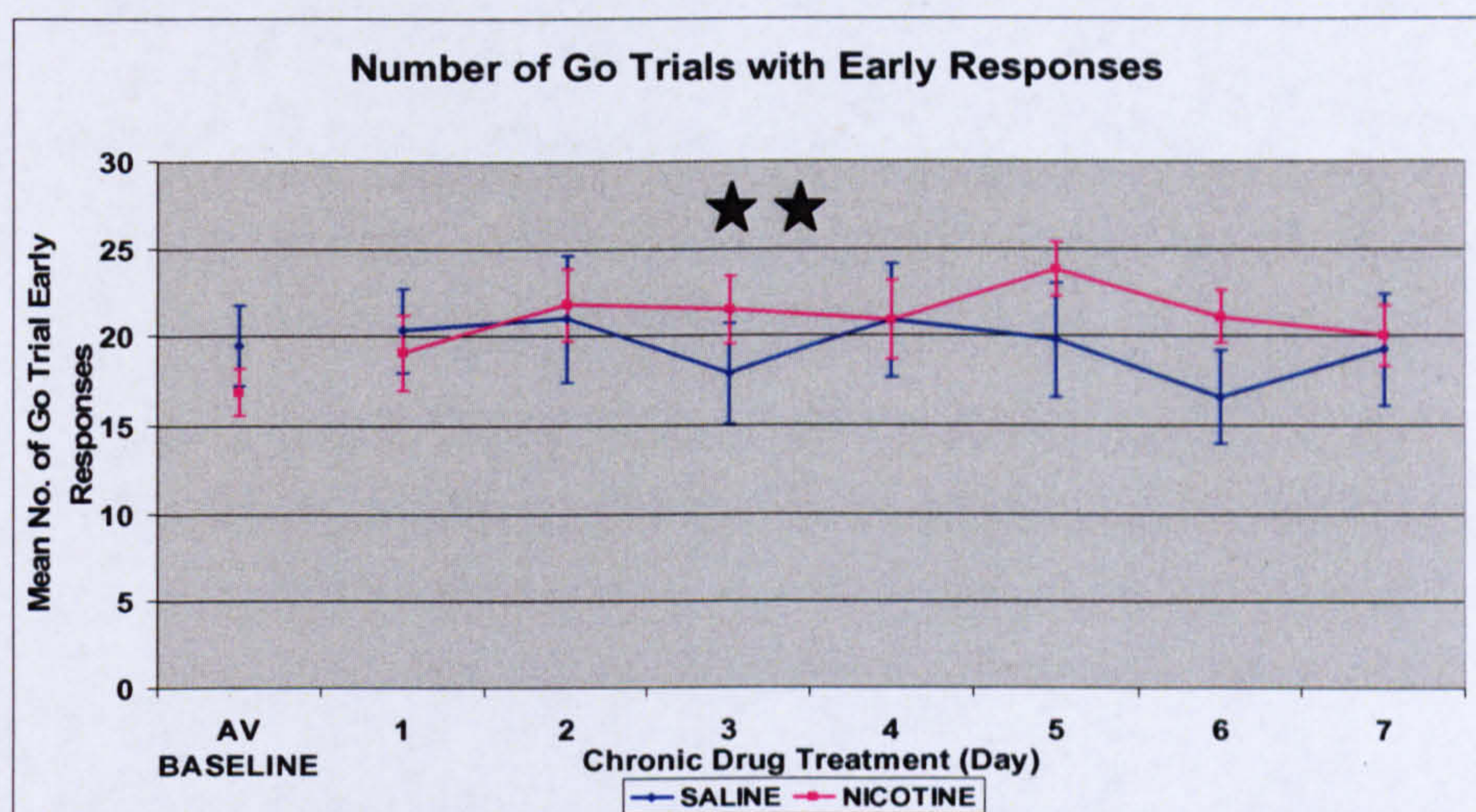
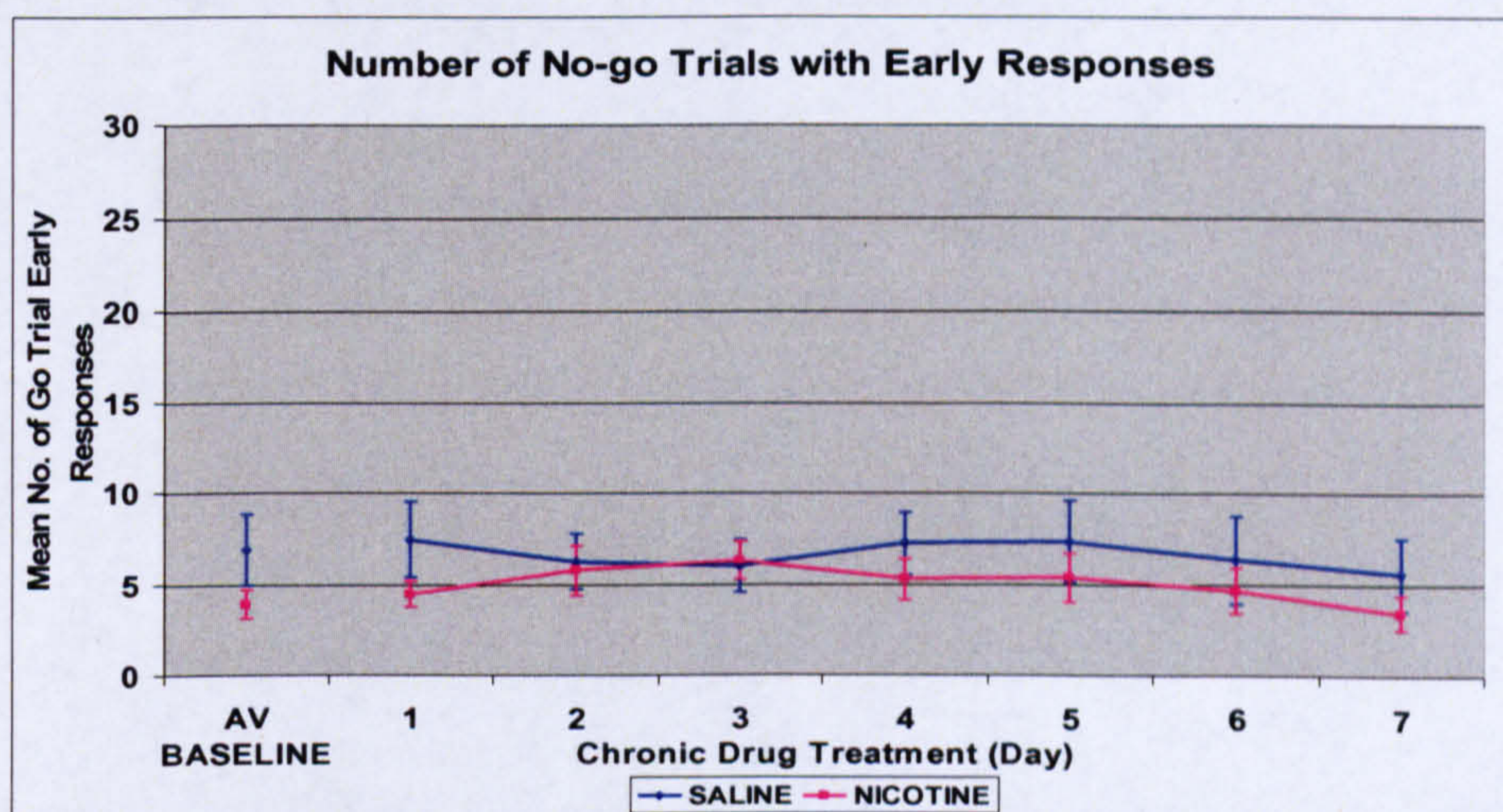
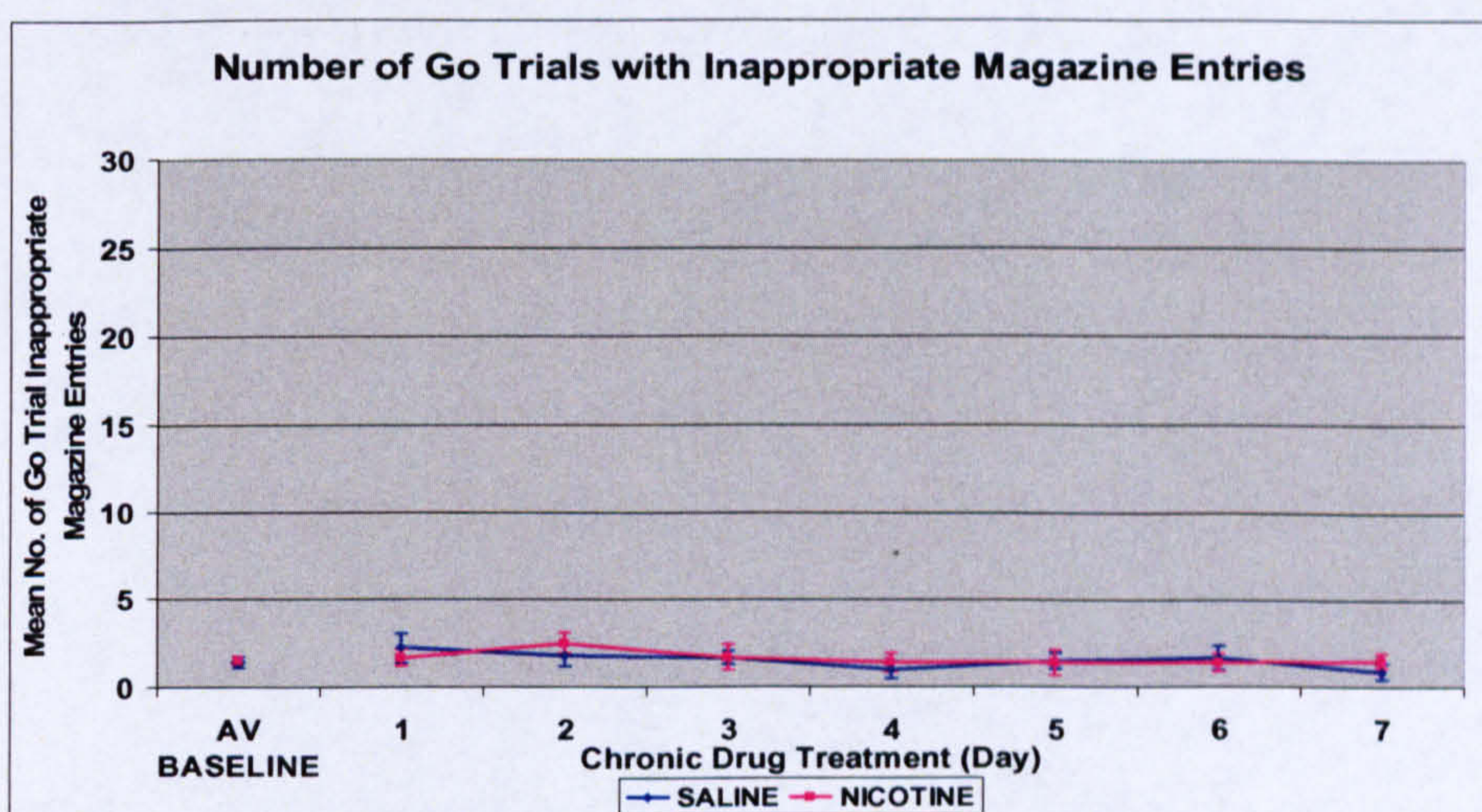
5.2.2.2.2. Anticipatory Responding

Analysis of anticipatory responding revealed that animals chronically treated with nicotine performed a significantly greater number of early responses during Go trials ($F(1,16) = 9.033, p = 0.008$) (Fig. 5.2(a)). In contrast, no significant main effects of treatment group were indicated for early responding during No-go trials or inappropriate magazine entries during Go and No-go trials (all $F(1,16) \leq 2.219, N.S.$). Analysis across all anticipatory measures during drug treatment furthermore demonstrated no significant group x test day interactions (all $F(6,96) \leq 1.386, N.S.$) (Fig. 5.2 (a-d)).

5.2.2.2.3. Speed of Responding

Nicotine treated animals latency to respond correctly during Go trials decreased in comparison to baseline latency (see Fig. 5.3 (a)). ANCOVA demonstrated a significant main effect of group ($F(1,16) = 4.798, p = 0.044$), with nicotine animals responding significantly faster than saline treated animals. No significant group x test day interaction was found ($F(6,96) = 0.386, N.S.$). In contrast incorrect response latencies during No-go trials did not differ between groups as shown by the non-significant main effect of group ($F(1,16) = 0.783, N.S.$) and group x test day interaction ($F(6,96) = 0.241, N.S.$).

Analysis of the latencies to collect the reward, demonstrated no differences between groups during both Go and No-go trials as indicated by the lack of significant main effects of group (all $F(1,16) \leq 0.208, N.S.$) and group x test day interactions (all $F(6,96) \leq 1.504, N.S.$) (See Fig. 5.3 (c-d)).

**Fig. 5.2 (a)****Fig. 5.2 (b)****Fig. 5.2 (c)**

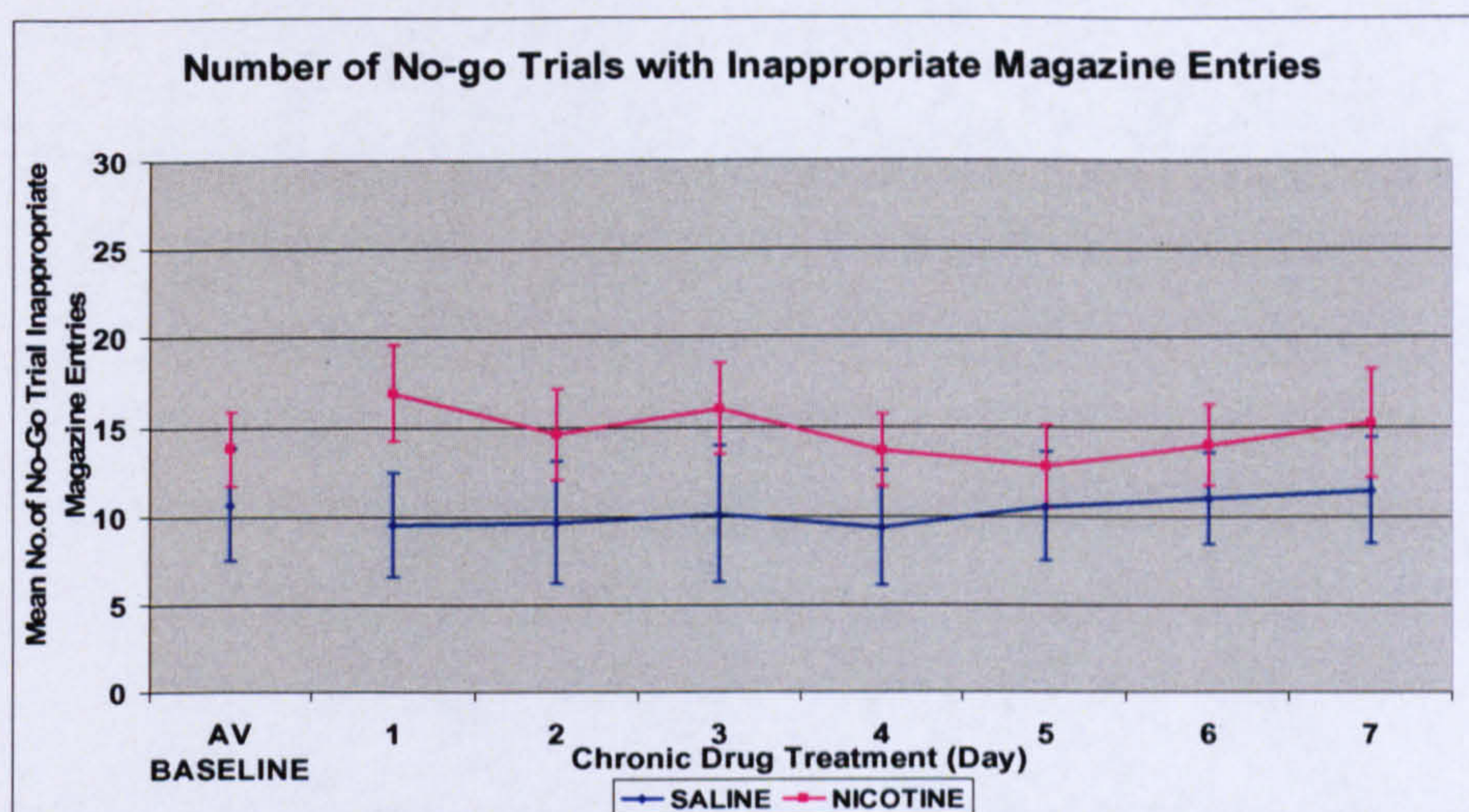
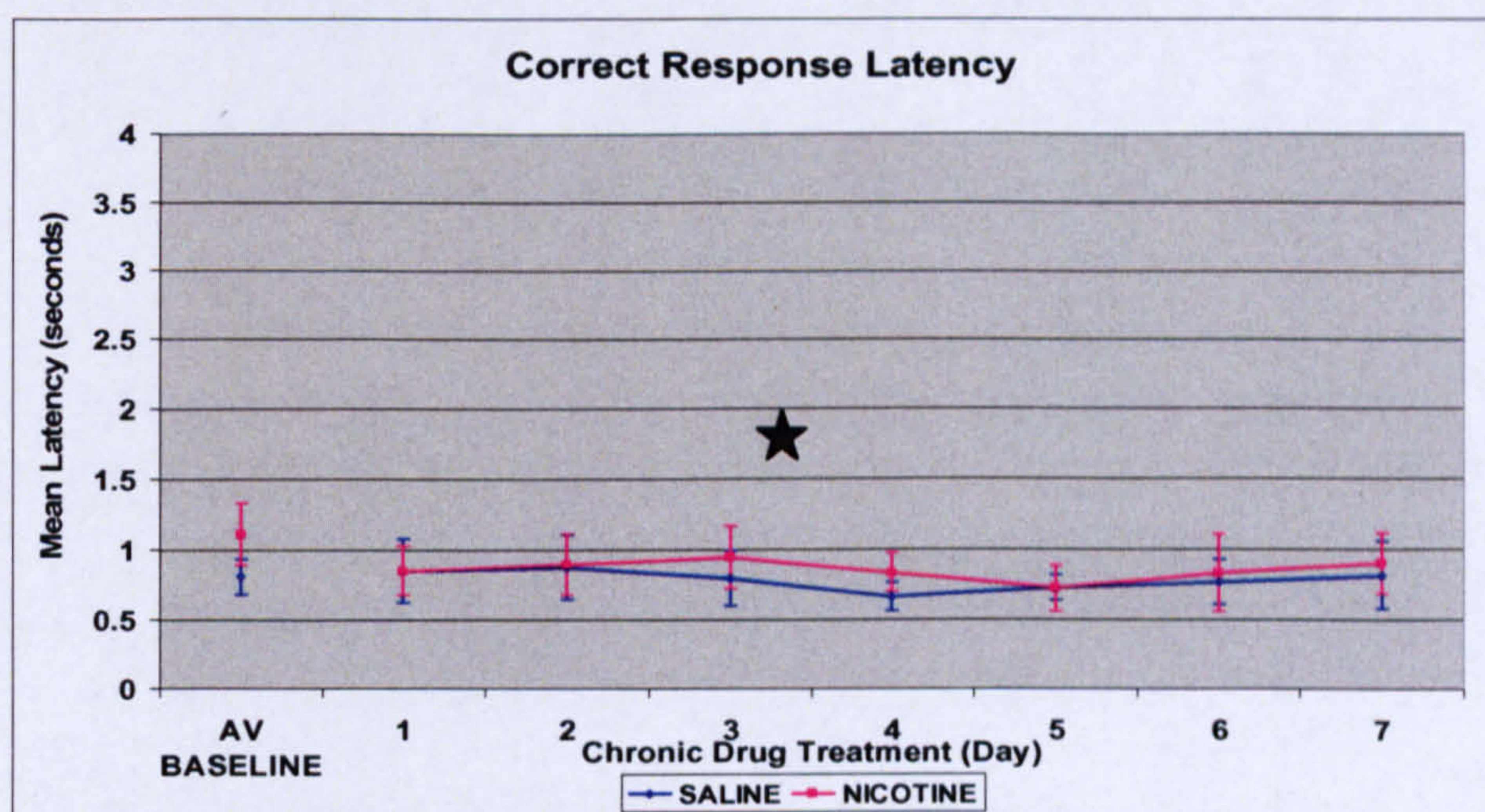
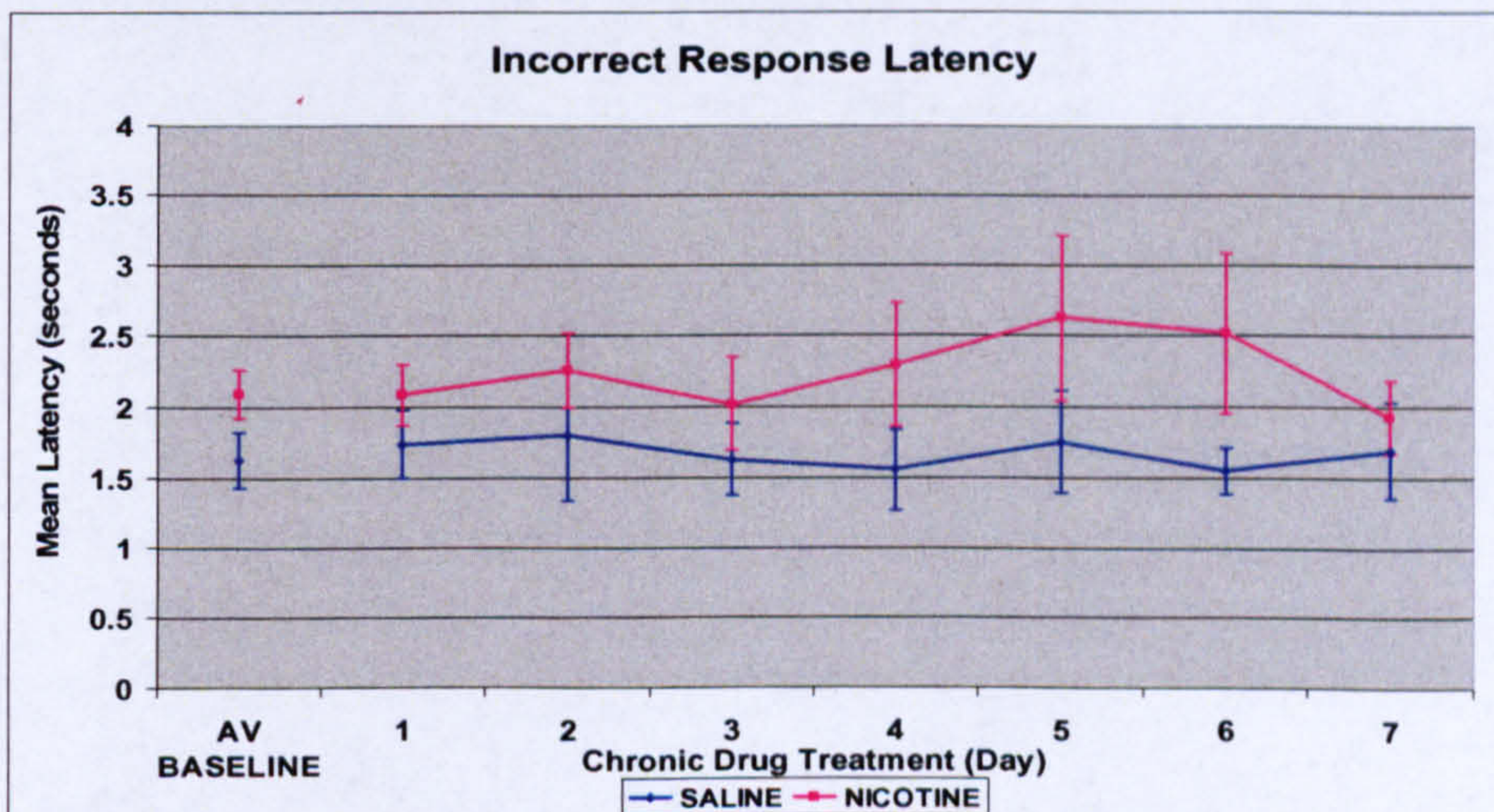
**Fig. 5.2 (d)**

Fig. 5.2 (a-d): Anticipatory Responding: effects of chronic nicotine administration on early responses during Go trial (a) and No-go trials (b). Anticipatory Responding: effects of chronic nicotine administration on inappropriate magazine entries during Go trials (c) and No-go trials (d). Each point represents the mean number of trials \pm SEM. ★★ $p < 0.01$ Main effect of group.

**Fig. 5.3 (a)****Fig. 5.3 (b)**

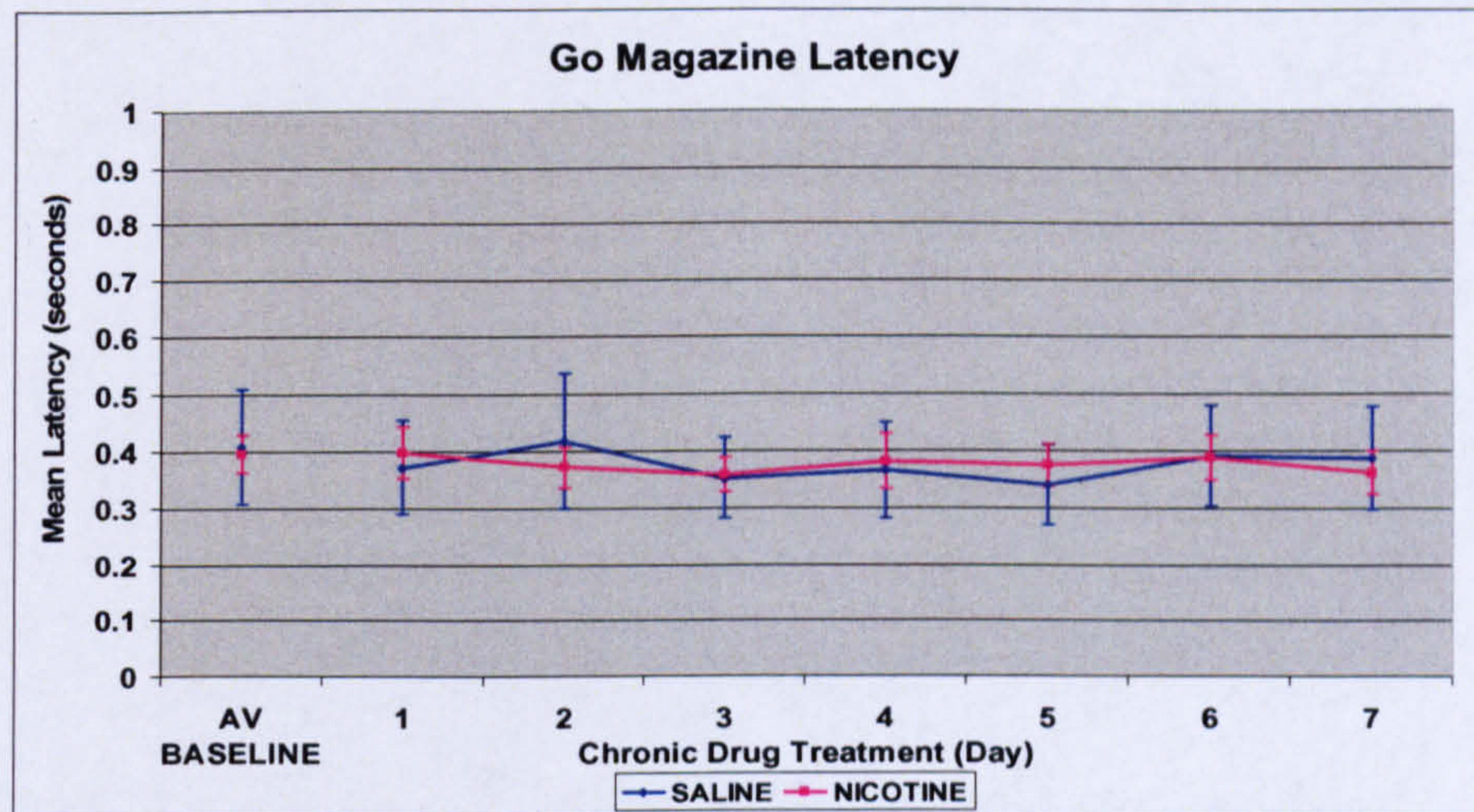


Fig. 5.3 (c)

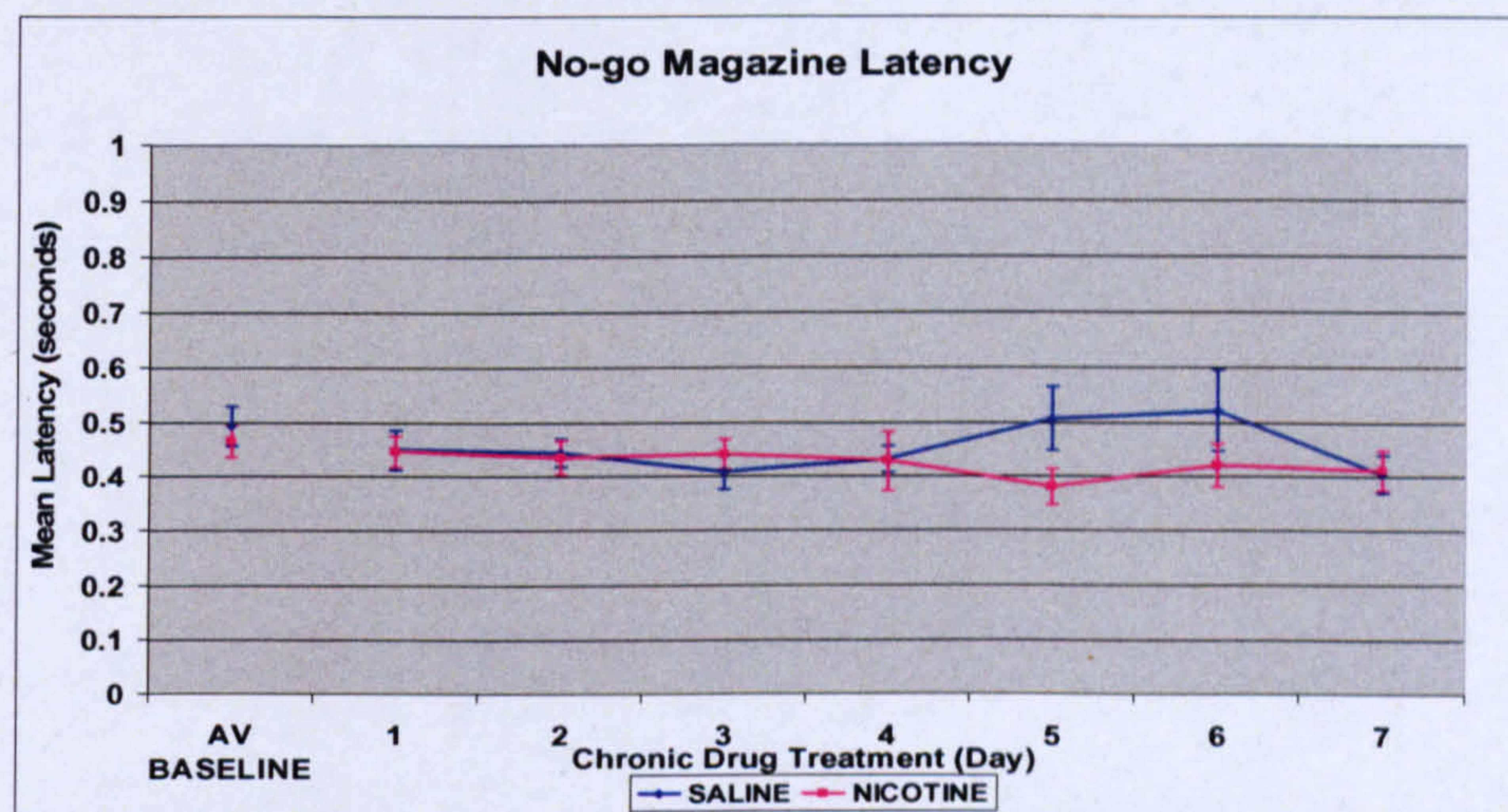


Fig. 5.3 (d)

Fig. 5.3 (a-d): Speed of Responding: the effects of chronic nicotine administration on latency in seconds to respond correctly during Go trials (a) and incorrectly during No-go trials (b). Speed of Responding: the effects of chronic nicotine administration on latency in seconds to collect reward following correct Go trials (c) and No-go Trials (d). Each point represents the mean latency in seconds \pm SEM. ★ $p < 0.05$ Main effect of group

5.2.2.3. NICOTINE WITHDRAWAL WEEK ONE

5.2.2.3.1. Accuracy of Performance

As clearly shown in Fig. 5.1 (a), total percentage of correct trials was consistently greater in animals experiencing nicotine withdrawal across sessions with the exception of 132 hours post termination of treatment. This observation was supported statistically by both a significant between group difference ($F(1,16) = 9.572$, $p = 0.007$) and group \times test day interaction ($F(8,128) = 2.222$, $p = 0.030$). Simple effects analysis demonstrated that nicotine treated animals exhibited a significantly higher overall performance at 12 hours post pump removal compared to saline treated animals ($F(1,16) = 0.967$, $p = 0.005$). Furthermore, the deficits in performance in animals experiencing nicotine withdrawal at 42 and 60 hours post termination of treatment just missed significance following Bonferroni adjustment ($F(1,16) = 4.852$, $p = 0.043$;

$F(1,16) = 7.642$, $p = 0.014$, respectively). One way ANCOVA by group revealed no significant main effect of test day for either the nicotine ($F(8,64) = 0.548$, N.S.) or saline treatment group ($F(8,56) = 0.513$, N.S.).

As illustrated in Fig. 5.1 (c) the pattern of performance on No-go trials across groups mirrors that of overall accuracy (see Fig 5.1 (a)), with animals in nicotine withdrawal almost consistently demonstrating a greater percentage of correct No-go trials, with the greatest effect observed at 12 hours of withdrawal. Both a significant main effect of group ($F(1,16) = 9.225$, $p = 0.008$) and group x test day interaction ($F(8,128) = 2.911$, $p = 0.005$) was revealed. Further analysis demonstrated that the decrease in impulsive responding during nicotine withdrawal reached significance at 12 and 60 hours post pump removal relative to the control group (all $F(1,16) \geq 9.113$ $p \leq 0.005$). Differences between groups in addition almost reached significance at 36, 42 and 156 hours post pump removal ($F(1,16) = 4.163$, $p = 0.058$; $F(1,16) = 4.484$, $p = 0.050$; $F(1,16) = 4.242$, $p = 0.056$, respectively). Further statistical exploration of the interaction revealed no significant main effect of test day for both treatment groups (saline, $F(8,56) = 0.489$, N.S.; nicotine, $F(8,64) = 8.10$, N.S.).

Finally in contrast there was no difference in the accuracy of performance of Go trials between groups (see Fig. 5.1 (b)), with an absence of both significant between group ($F(1,17) = 0.189$, N.S.) and group x test day interactions found ($F(8,16) = 0.503$, N.S.).

5.2.2.3.2. Anticipatory Responding

As tabulated in Table 5.3 the frequency of early responses during both Go and No-go trials were lower in animals experiencing nicotine withdrawal. After controlling however for base line differences in early responding no significant between group (all $F(1,16) \leq 2.176$, N.S.) or group x test day interactions (all $F(8,128) \leq 1.004$, N.S.) were found.

Analysis of inappropriate magazine entries once again revealed no differences between treatment groups during both types of trials, as indicated by the lack of main effects of group (all $F(1,16) \leq 0.559$, N.S.) and group x test day interactions (all $F(8,128) \leq 0.506$, N.S.) (see Table 5.3).

5.2.2.3.3. Speed of Responding

Table 5.4 summarises the effects of spontaneous withdrawal on speed of responding. Across week one of withdrawal the speed with which animals responded on the lever during both Go and No-go trials did not differ across groups, with non-significant group effects (all $F(1,16) \leq 0.094$, N.S.) and group x test day interactions found (all $F(8,128) \leq 1.583$, N.S.).

Withdrawal did however differentially affect the latencies to collect the reward depending upon the type of trial being performed. During No-go trials there was no difference between groups in speed to collect the reward, supported statistically by the non-significant main effect of group ($F(1,16) = 0.132$, N.S.) and group x day interaction observed ($F(8,128) = 0.583$, N.S.). In contrast, whilst no main effect of group was shown for Go trial magazine latencies ($F(1,16) = 2.389$, N.S.), a significant group x test day interaction was displayed ($F(8,128) = 3.230$, $p = 0.002$). Further analysis however demonstrated no significant differences between treatment groups on latency to collect reward during Go trials (all $F(1,16) \leq 7.084$, N.S.). Independent examination of treatment groups furthermore revealed no significant main effects of test day for either nicotine ($F(8,64) = 0.322$, N.S.) or saline treated animals ($F(8,56) = 0.838$, N.S.).

5.2.2.3.4. Somatic Withdrawal Signs

As shown in Fig 5.4, animals experiencing nicotine withdrawal displayed a sharp elevation in the total frequency of somatic signs beginning at 6 hours post termination of drug treatment returning to baseline at 180.5 hours. Two-way ANOVA demonstrated both a significant main effect of treatment group ($F(1, 17) = 8.208$, $p = 0.011$) and group x time interaction ($F(13, 221) = 3.806$, $p < 0.001$). Further analysis demonstrated that the somatic signs exhibited by animals in nicotine withdrawal were significantly elevated in comparison to saline treated animals at 24, 36.5, 42.5, 84.5 hours post pump removal (all $t \geq 3.420$, $df = 17$, $p \leq 0.003$). Furthermore, elevation of total somatic signs in animals experiencing nicotine withdrawal just missed significance at 12.5, 18.5, 60.5 and 156.5 hours post pump removal following Bonferroni correction (all $t \geq 2.127$, $df = 17$, $p \leq 0.030$).

Importantly no significant differences between treatment groups were displayed in the frequency of symptoms during baseline or chronic drug treatment (all $t \leq 0.260$, $df = 17$, N.S.). Further support of the existence of the spontaneous exhibition of the nicotine withdrawal syndrome is evident by the highly significant main effect of time shown in the nicotine treated animals ($F(13, 117) = 16.298$, $p < 0.001$) in contrast to the saline treated group ($F(13, 104) = 2.345$, N.S.). The saline treated animals displayed no evidence of an increase in frequency of overall somatic signs during withdrawal in comparison to baseline.

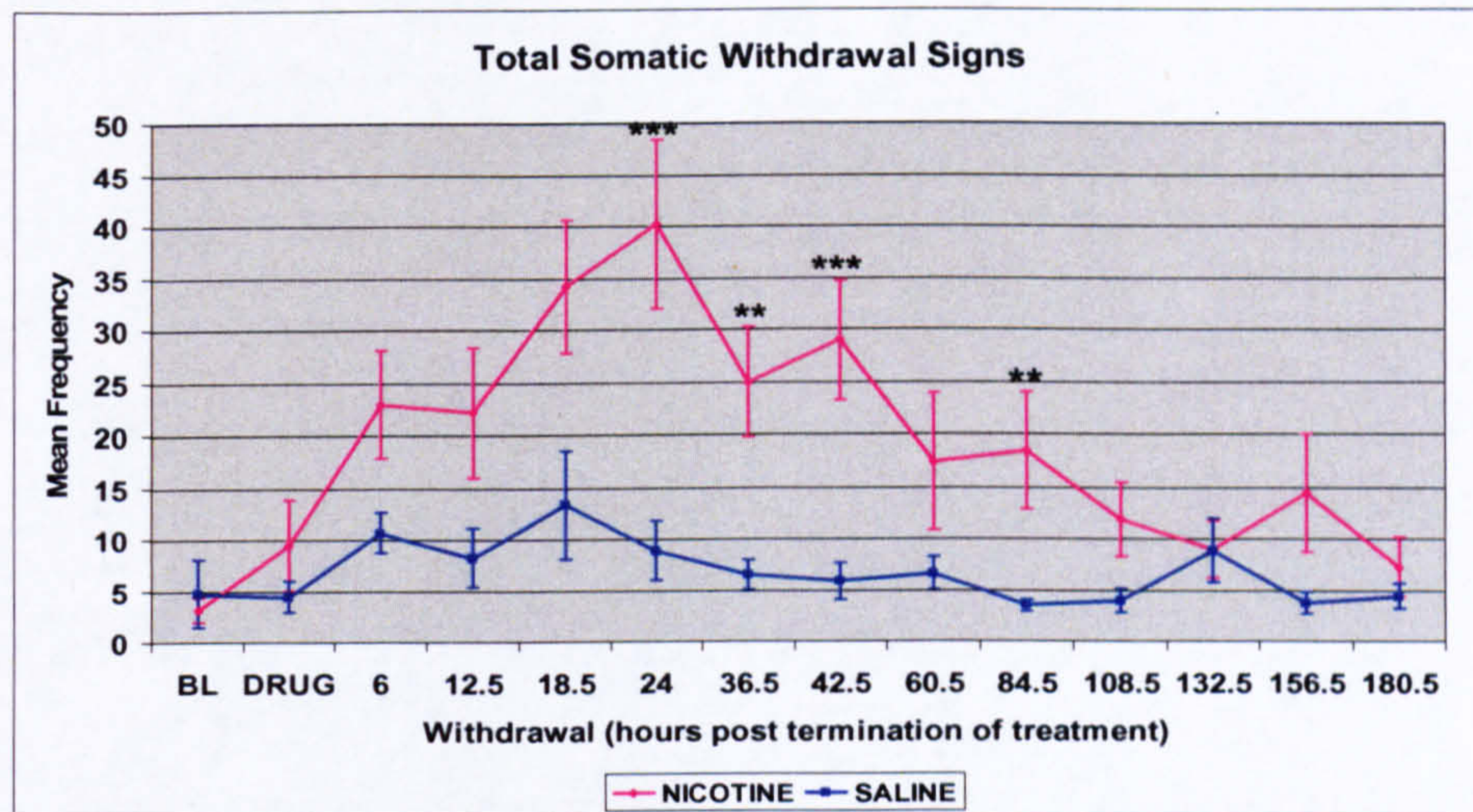


Fig. 5.4: Total Somatic Withdrawal Signs: the frequency of overall somatic signs displayed during spontaneous withdrawal. Each point represents the mean number of trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) indicate significant differences between nicotine and saline treatment groups.

Table 5.5 displays the frequency of individual categories of somatic signs observed during spontaneous withdrawal. One of the most commonly exhibited category of symptoms in animals experiencing nicotine withdrawal were teeth chattering and chews. The increase in frequency reached significance in comparison to saline treated animals as early as 6 hours post pump removal and continued for a time course of 132.5 hours (all $U \geq 13.50$, $N = 19$, $p \leq 0.022$). In terms of gasps and writhes, animals experiencing nicotine withdrawal more frequently displayed these somatic signs in comparison to saline treated animals at 18.5 and 42.5 hours post termination of treatment (all $U \geq 27.50$, $N = 19$, $p \leq 0.039$). Shakes and tremors furthermore showed a significant increase in nicotine treated animals at 18.5 and 36.5 hours of spontaneous withdrawal (all $U \geq 21.00$, $N = 19$, $p \leq 0.047$). Finally, the miscellaneous observational category, including signs for example of scratching and foot licks, elevated significantly in nicotine treated animals at 18.5, 24, 36.5, 42.5, 84.5 and 156.5 hours post pump removal (all $U \geq 3.857$, $N = 19$, $p \leq 0.05$).

Table 5.3: Anticipatory responding in the symmetrically reinforced go/no-go task during withdrawal week one

Behavioural Measure		12 hours	18 hours	36 hours	42 hours	60 hours	84 hours	108 hours	132 hours	156 hours
Go trials with early responses	Nicotine	14.80 ± 1.75	15.30 ± 2.31	17.40 ± 2.20	16.90 ± 2.53	15.60 ± 1.57	18.80 ± 1.60	16.10 ± 1.80	18.80 ± 1.79	16.90 ± 1.62
	Saline	19.11 ± 3.20	20.56 ± 2.61	19.67 ± 2.72	19.33 ± 2.99	19.89 ± 2.90	19.44 ± 2.32	21.78 ± 3.00	18.22 ± 3.30	19.89 ± 3.22
No-go trials with early responses	Nicotine	1.70 ± 0.45	3.10 ± 1.07	2.80 ± 0.80	3.00 ± 0.67	3.50 ± 1.04	4.10 ± 0.94	2.80 ± 0.70	3.60 ± 0.90	3.30 ± 0.63
	Saline	6.67 ± 2.36	6.00 ± 1.98	6.67 ± 2.14	7.22 ± 2.33	7.44 ± 2.13	8.00 ± 1.94	8.11 ± 2.68	5.67 ± 1.44	7.78 ± 2.71
Go trials with magazine entries	Nicotine	1.30 ± 0.45	1.30 ± 0.54	1.00 ± 0.33	1.00 ± 0.33	1.30 ± 0.45	2.00 ± 0.63	1.60 ± 0.50	2.90 ± 1.31	1.60 ± 0.72
	Saline	1.44 ± 0.47	1.44 ± 0.58	2.11 ± 0.72	1.67 ± 0.44	1.89 ± 0.68	1.67 ± 0.75	1.44 ± 0.41	1.67 ± 0.62	1.33 ± 0.44
No-go trials with Magazine entries	Nicotine	13.30 ± 2.51	13.90 ± 2.64	14.90 ± 2.12	13.30 ± 1.91	16.40 ± 2.76	15.30 ± 2.42	14.30 ± 2.37	16.50 ± 2.75	15.50 ± 2.25
	Saline	12.89 ± 3.23	12.00 ± 3.03	14.78 ± 3.45	11.44 ± 3.07	12.56 ± 3.52	12.56 ± 3.59	12.56 ± 3.34	12.22 ± 2.57	13.56 ± 3.23

Table 5.3: Each value represents the mean frequency ± SEM.

Table 5.4: Speed of responding in the symmetrically reinforced go/no-go task during withdrawal week one

Behavioural Measure		12 hours	18 hours	36 hours	42 hours	60 hours 3	84 hours	108 hours	132 hours	156 hours
Correct response latency	Nicotine	1.04 ± 0.21	1.10 ± 0.38	0.92 ± 0.25	0.88 ± 0.25	0.96 ± 0.31	1.14 ± 0.41	1.10 ± 0.31	1.08 ± 0.41	0.95 ± 0.28
	Saline	0.68 ± 0.12	0.68 ± 0.14	0.87 ± 0.23	0.75 ± 0.18	0.70 ± 0.12	0.70 ± 0.12	0.68 ± 0.17	0.87 ± 0.22	0.64 ± 0.13
Incorrect response latency	Nicotine	1.61 ± 0.31	1.74 ± 0.24	1.97 ± 0.21	2.09 ± 0.60	1.69 ± 0.32	1.85 ± 0.33	1.76 ± 0.26	2.23 ± 0.40	1.96 ± 0.26
	Saline	1.35 ± 0.24	1.94 ± 0.35	1.90 ± 0.57	1.90 ± 0.43	1.79 ± 0.32	1.47 ± 0.35	1.72 ± 0.32	1.42 ± 0.35	1.75 ± 0.39
Go magazine latency	Nicotine	0.47 ± 0.07	0.45 ± 0.06	0.45 ± 0.07	0.40 ± 0.05	0.37 ± 0.04	0.42 ± 0.04	0.35 ± 0.04	0.39 ± 0.04	0.34 ± 0.04
	Saline	0.38 ± 0.07	0.34 ± 0.07	0.34 ± 0.07	0.34 ± 0.07	0.36 ± 0.08	0.42 ± 0.09	0.35 ± 0.11	0.33 ± 0.07	0.35 ± 0.09
No-go magazine latency	Nicotine	0.44 ± 0.05	0.47 ± 0.08	0.42 ± 0.05	0.40 ± 0.04	0.42 ± 0.04	0.43 ± 0.04	0.43 ± 0.06	0.44 ± 0.06	0.45 ± 0.08
	Saline	0.50 ± 0.05	0.44 ± 0.07	0.40 ± 0.05	0.39 ± 0.04	0.50 ± 0.11	0.46 ± 0.06	0.45 ± 0.10	0.47 ± 0.06	0.44 ± 0.08

Table 5.4: Each value represents the mean latency (seconds) ± SEM.

Table 5.5: Somatic signs of spontaneous withdrawal during withdrawal week one

Behavioural Measure		Hours Post Termination of Treatment													
		BL	Drug	6 hours	12.5 hours	18.5 hours	24 hours	36.5 hours	42.5 hours	60.5 hours	84.5 hours	108.5 hours	132.5 hours	156.5 hours	180.5 hours
Gasps and writhes	Nicotine	0.00	0.00	2.70	0.80	0.50*	1.00	0.00	0.90*	0.40	0.10	0.20	0.00	0.00	0.00
		±0.00	±0.00	±2.17	±0.33	±0.22	±0.60	±0.00	±0.59	±0.01	±0.02	±0.00	±0.00	±0.00	±0.00
	Saline	0.00	0.00	0.11	0.33	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		±0.00	±0.00	±0.33	±0.71	±0.00	±0.00	±0.33	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
Teeth chatters and chews	Nicotine	0.10	0.10	3.60*	2.00*	4.30*	3.30**	2.10**	3.50	2.60	1.30	1.40*	0.70	0.50	0.20
		±0.10	±0.10	±1.08	±0.67	±1.48	±0.83	±0.87	±1.26	±1.29	±0.65	±0.56	±0.33	±0.27	±0.20
	Saline	0.00	0.00	0.67	0.22	0.56	0.11	0.00	1.11	0.56	0.22	0.22	0.33	0.11	0.00
		±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
Shakes and Tremors	Nicotine	0.00.	1.00	3.10	1.80	3.00*	3.40	1.50*	1.80	1.30	0.90	0.60	0.20	0.10	0.20
		±0.00	±0.39	±0.46	±0.44	±0.63	±0.97	±0.17	±0.61	±0.30	±0.38	±0.22	±0.13	±0.10	±0.13
	Saline	0.22	1.44	2.00	1.44	1.22	1.11	0.89	0.56	1.11	0.44	0.44	0.33	0.56	0.67
		±0.22	±0.71	±0.41	±0.47	±0.36	±0.39	±0.31	±0.24	±0.48	±0.24	±0.18	±0.17	±0.18	±0.24
Miscellaneous	Nicotine	3.10	8.40	13.60	17.60	26.40*	32.50**	21.40*	22.80**	13.10	16.10**	9.70	8.10	13.80*	6.90
		±1.22	±4.39	±4.77	±6.25	±6.16	±8.87	±5.47	±5.14	±6.44	±5.50	±3.04	±2.51	±5.56	±2.83
	Saline	4.56	3.00	7.89	6.22	11.56	7.67	5.56	4.22	5.00	2.89	3.33	8.22	3.11	3.77
		±3.33	±0.88	±1.92	±2.82	±5.04	±2.61	±1.34	±1.46	±1.58	±0.45	±1.14	±3.13	±0.92	±1.09

Table 5.5 : Somatic Withdrawal Signs: the frequency of somatic signs displayed during spontaneous withdrawal. Each value represents the mean frequency (seconds) ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 indicate significant differences between nicotine and saline treatment groups.

5.2.2.4. NICOTINE WITHDRAWAL WEEK TWO

5.2.2.4.1. Accuracy of Performance

As shown in Fig. 5.1 (a) overall performance in the task during the second week of withdrawal did not differ across groups, as demonstrated by the lack of significant group effect ($F(1,16) = 1.756$, N.S.) and group x test day interaction found ($F(6,96) = 1.183$, N.S.). Analysis of the accuracy of performance during go and no-go trials produced similar results, with main effects of treatment group (Go trials, $F(1,17) = 1.933$, N.S.; No-go trials, $F(1,16) = 0.176$, N.S.) and group x test day interactions failing to reach significance (Go trials, $F(6,102) = 0.819$, N.S.; No-Go trials, $F(6,96) = 1.745$, N.S.) (see Fig. 5.1 (b-c)). Although no statistically significant differences were found between groups during performance on No-go trials, as clearly illustrated in Fig. 5.1 (c) from the 9th day of withdrawal there was a steady decrease in accuracy of performance of No-go trials displayed by the chronically treated nicotine group.

5.2.2.4.2. Anticipatory Responding

Minimal difference across groups was observed in the frequency of early responses performed during both Go and No-go trials. No significant between group (all $F(1,16) \leq 0.337$, N.S.) or group x test day interactions (all $F(6,96) \leq 1.816$, N.S.) were demonstrated on this anticipatory measure (see Fig. 5.5 (a-b)).

In contrast nicotine treated animals consistently displayed a greater number of magazine entries compared to the saline treated animals during Go trials ($F(1,16) = 5.210$, $p = 0.036$) (Fig. 5.5 (c)). The difference observed did not interact with test day ($F(6, 96) = 0.199$, N.S.). Analysis of magazine entries during No-go trials did not demonstrate a main effect of group ($F(1,16) = 0.027$, N.S.), although a significant group x test day interaction was however shown ($F(6,96) = 2.305$, $p = 0.04$). Despite a tendency for this anticipatory behaviour to be more frequent in animals treated previously with nicotine, simple effects analysis failed to demonstrate any significant differences between groups across test days (all $F(1,16) \leq 2.412$, N.S.) (see Fig. 5.5 (d)). Furthermore no significant main effect of test day was shown for either the previously treated nicotine ($F(6,48) = 0.365$, N.S.) or saline animals ($F(6,42) = 0.669$, N.S.).

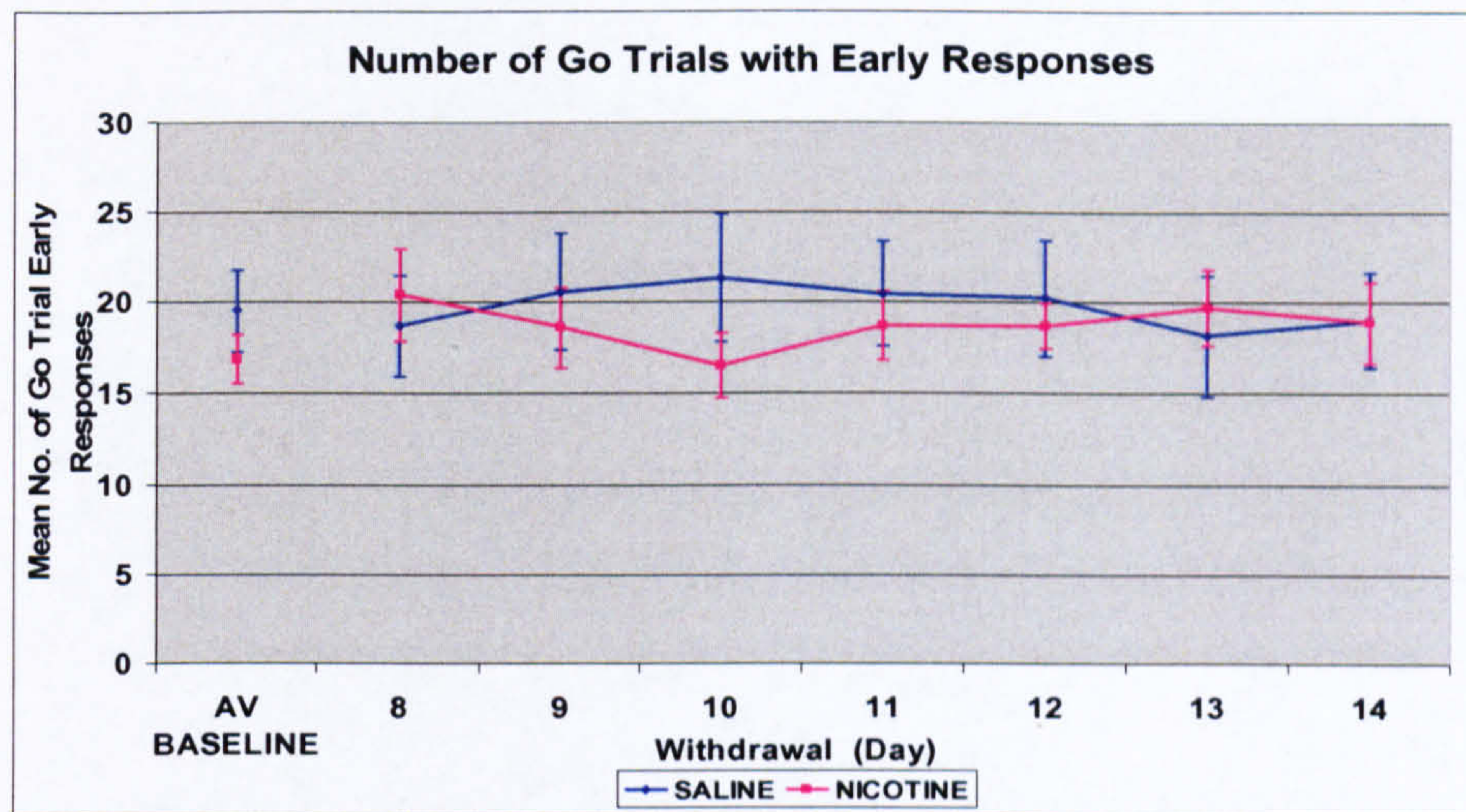


Fig. 5.5 (a)

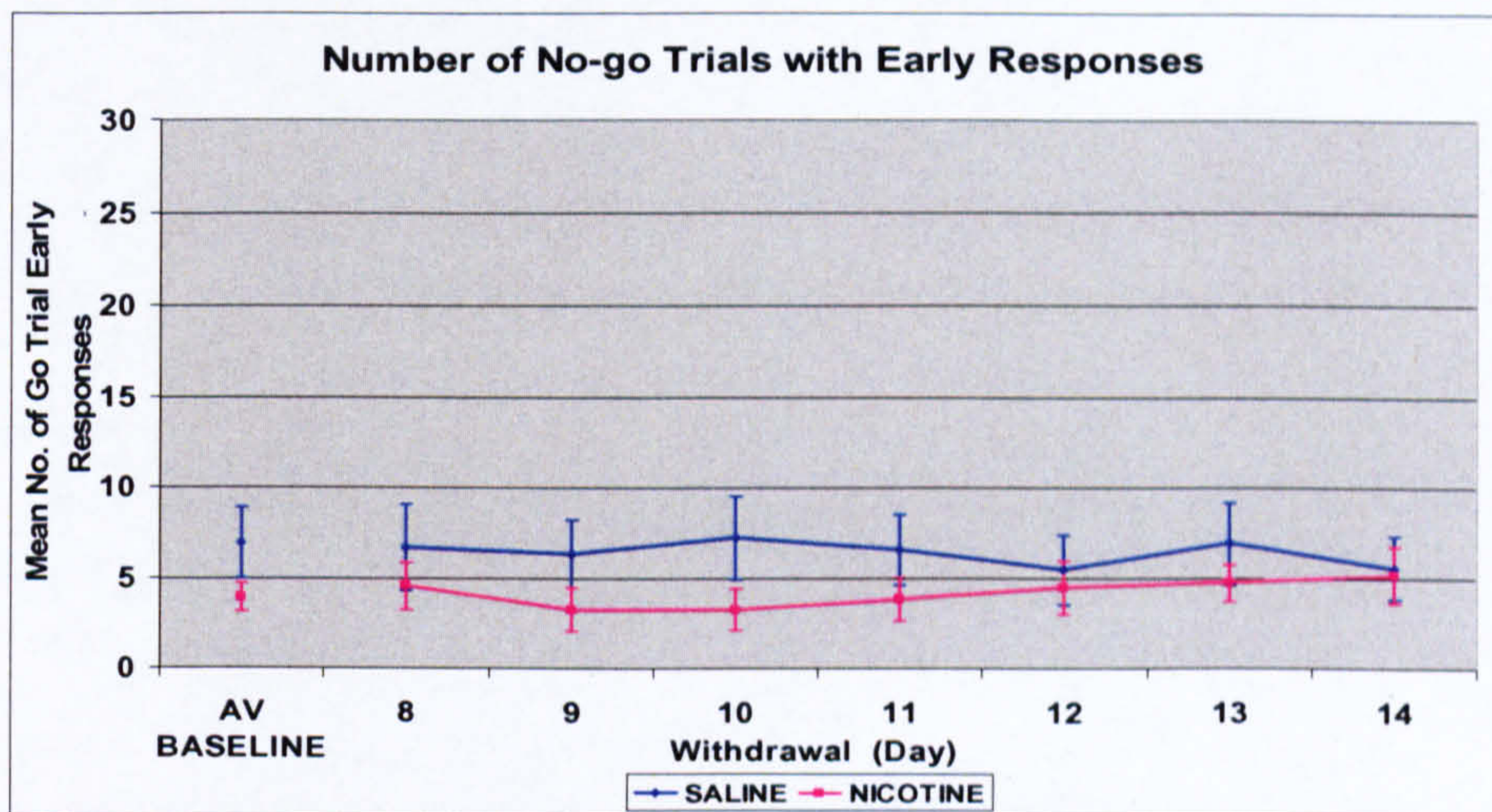


Fig. 5.5 (b)

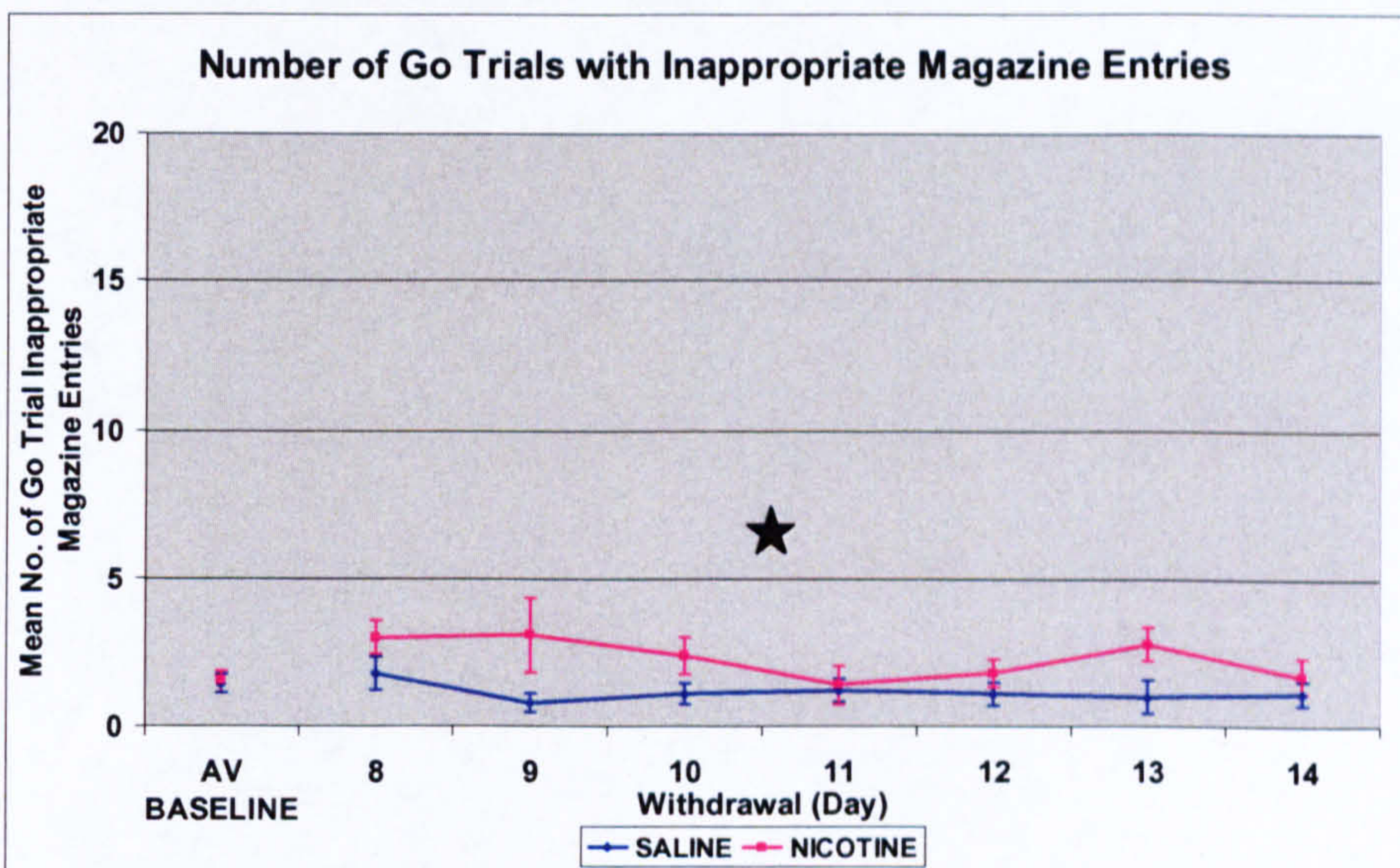


Fig. 5.5 (c)

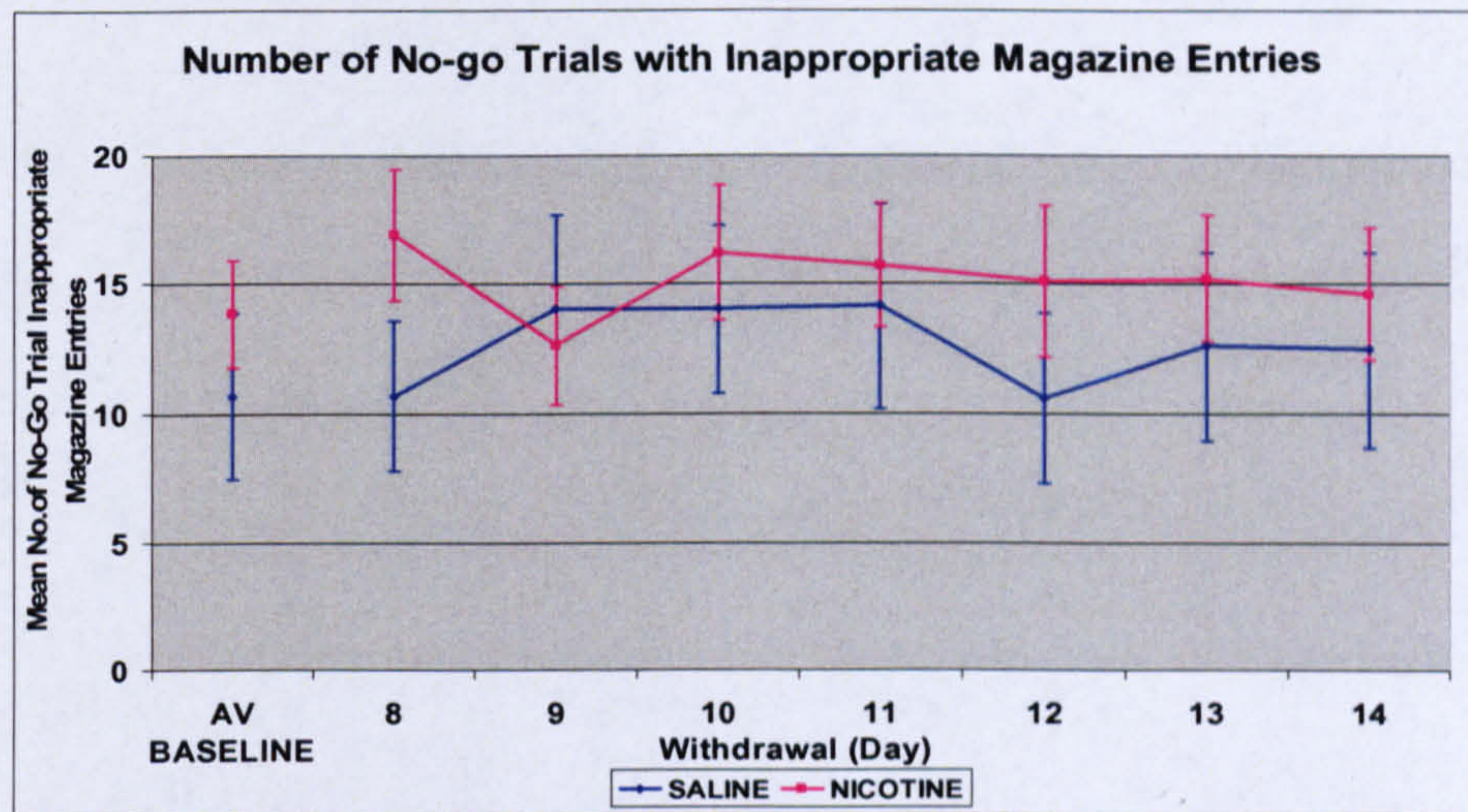


Fig. 5.5 (d)

Fig. 5.5 (a-d): Anticipatory Responding: effects of nicotine withdrawal during the second week post termination of treatment on early responses during Go trials (a) and No-go trials (b). Anticipatory Responding: effects of nicotine withdrawal during the second week post termination of treatment on inappropriate magazine entries during Go trials (c) and No-go trials (d). Each point represents the mean number of trials \pm SEM. ★ $p < 0.05$ Main effect of group

5.2.2.4.3. Speed of Responding

Both correct and incorrect response latencies did not differ across groups as demonstrated by the non-significant between group effects (all $F(1,16) \leq 0.035$, N.S.) and group \times test day interactions (all $F(6,96) \leq 1.726$, N.S.).

During the second week of withdrawal latency to collect the reward following both correct Go and No-go trials was similar across groups. ANCOVA revealed a non-significant effect of group (all $F(1,16) \leq 0.117$, N.S.) and group \times day interaction (all $F(6,96) \leq 0.444$, N.S.) on both measures. Analysis of Go magazine latencies did however demonstrate a significant main effect of test day irrespective of treatment group ($F(6,96) = 3.130$, $p = 0.038$). Post hoc comparisons however revealed no significant differences between tests sessions. Table 5.6 summaries speed responding measures across withdrawal week two.

5.2.2.5. NICOTINE WITHDRAWAL WEEK THREE

5.2.2.5.1. Accuracy of Performance

No group differences were observed on overall accuracy of performance during the third week of testing following the termination of chronic drug treatment, as indicated by the lack of significant between group ($F(1,16) = 2.326$, N.S.) and group \times test day interaction ($F(6,96) = 1.979$, N.S.) (see Fig. 5.1 (a)). Independent analysis of Go trials demonstrated parallel findings with an absence of group differences, evident by a non-significant group effect ($F(1,17) = 0.617$, N.S.), and group \times test day interaction ($F(2.447, 41.601) = 1.618$, N.S.) (see Fig. 5.1 (b)). However, as illustrated in Fig. 5.1 (c) performance on No-go trials in animals previously treated

with nicotine displayed a gradual decline in accuracy of performance from the 9th day post termination of treatment. This deficit in performance continued during the early stages of the third week of nicotine withdrawal, as supported by the significant group x test day interaction ($F(6,96) = 2.924, p = 0.012$). Further analysis revealed that the increase in behavioural disinhibition expressed by the nicotine treated group reached significance on day 17th day post chronic drug treatment ($F(1,16) = 13.271, p = 0.002$), whilst day 15 just failed to reach significance following Bonferroni correction ($F(1,16) = 4.829, p = 0.043$). A one way ANCOVA of test day revealed no significant main effects for both the saline ($F(6,42) = 0.503, N.S.$) and nicotine treated rats ($F(6,48) = 1.504, N.S.$) across this seven day period.

5.2.2.5.2. Anticipatory Responding

Table 5.7 summarises anticipatory responding measures across treatment groups in the third week of withdrawal. No difference in responding within the task was shown between treatment groups across all measures of anticipatory responding. A lack of both significant group effects (all $F(1,16) \leq 0.575, N.S.$) and group x test day interactions (all $F(6,96) \leq 1.292, N.S.$) were displayed following ANCOVA of early responding and magazine entries during both Go and No-go trials.

5.2.2.5.3. Speed of Responding

Analysis of both incorrect and correct response latencies and the speed at which reward was collected during both Go and No-go trials demonstrated no significant differences between groups (all $F(1,16) \leq 3.021, N.S.$) or interactions of group performance across test sessions (all $F(6,96) \leq 1.643, N.S.$). Speed of responding measures across treatment groups are summarised in Table 5.8.

Table 5.6: Speed of responding in the symmetrically reinforced go/no-go task during withdrawal week two

Behavioural Measure		Day 8	Day 9	Day10	Day 11	Day 12	Day 13	Day 14
Correct response latency	Nicotine	0.96 ± 0.31	1.14 ± 0.34	1.04 ± 0.30	1.06 ± 0.29	0.96 ± 0.25	1.00 ± 0.22	0.89 ± 0.15
	Saline	0.74 ± 0.16	0.80 ± 0.26	0.76 ± 0.22	0.67 ± 0.15	0.85 ± 0.21	0.95 ± 0.21	0.86 ± 0.18
Incorrect response Latency	Nicotine	2.38 ± 0.36	1.71 ± 0.37	2.29 ± 0.39	2.30 ± 0.58	2.32 ± 0.47	2.73 ± 0.54	2.42 ± 0.61
	Saline	1.51 ± 0.35	1.94 ± 0.35	1.66 ± 0.33	1.57 ± 0.26	1.72 ± 0.25	1.96 ± 0.38	2.41 ± 0.39
Go magazine latency	Nicotine	0.35 ± 0.06	0.36 ± 0.04	0.35 ± 0.03	0.33 ± 0.03	0.36 ± 0.04	0.34 ± 0.03	0.34 ± 0.03
	Saline	0.32 ± 0.06	0.36 ± 0.08	0.35 ± 0.07	0.36 ± 0.09	0.36 ± 0.09	0.35 ± 0.09	0.37 ± 0.08
No-go magazine latency	Nicotine	0.40 ± 0.03	0.40 ± 0.05	0.41 ± 0.06	0.39 ± 0.03	0.46 ± 0.10	0.37 ± 0.04	0.40 ± 0.03
	Saline	0.48 ± 0.06	0.42 ± 0.07	0.48 ± 0.06	0.44 ± 0.09	0.47 ± 0.09	0.42 ± 0.06	0.44 ± 0.06

Table 5.6: Each value represents the mean latency (seconds) ± SEM.

Table 5.7: Anticipatory responding in the symmetrically reinforced go/no-go task during withdrawal week three

Behavioural Measure		Day 8	Day 9	Day10	Day 11	Day 12	Day 13	Day 14
Correct response latency	Nicotine	0.96 ± 0.31	1.14 ± 0.34	1.04 ± 0.30	1.06 ± 0.29	0.96 ± 0.25	1.00 ± 0.22	0.89 ± 0.15
	Saline	0.74 ± 0.16	0.80 ± 0.26	0.76 ± 0.22	0.67 ± 0.15	0.85 ± 0.21	0.95 ± 0.21	0.86 ± 0.18
Incorrect response latency	Nicotine	2.38 ± 0.36	1.71 ± 0.37	2.29 ± 0.39	2.30 ± 0.58	2.32 ± 0.47	2.73 ± 0.54	2.42 ± 0.61
	Saline	1.51 ± 0.35	1.94 ± 0.35	1.66 ± 0.33	1.57 ± 0.26	1.72 ± 0.25	1.96 ± 0.38	2.41 ± 0.39
Go magazine latency	Nicotine	0.35 ± 0.06	0.36 ± 0.04	0.35 ± 0.03	0.33 ± 0.03	0.36 ± 0.04	0.34 ± 0.03	0.34 ± 0.03
	Saline	0.32 ± 0.06	0.36 ± 0.08	0.35 ± 0.07	0.36 ± 0.09	0.36 ± 0.09	0.35 ± 0.09	0.37 ± 0.08
No-go magazine latency	Nicotine	0.40 ± 0.03	0.40 ± 0.05	0.41 ± 0.06	0.39 ± 0.03	0.46 ± 0.10	0.37 ± 0.04	0.40 ± 0.03
	Saline	0.48 ± 0.06	0.42 ± 0.07	0.48 ± 0.06	0.44 ± 0.09	0.47 ± 0.09	0.42 ± 0.06	0.44 ± 0.06

Table 5.7: Each value represents the mean frequency ± SEM.

Table 5.8: Speed of responding in the symmetrically reinforced go/no-go task during withdrawal week three

Behavioural Measure		Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Correct response latency	Nicotine	0.91 ± 0.21	0.91 ± 0.16	0.81 ± 0.15	1.01 ± 0.21	0.97 ± 0.24	1.13 ± 0.28	1.00 ± 0.23
	Saline	0.78 ± 0.14	0.87 ± 0.18	0.91 ± 0.27	0.85 ± 0.19	0.82 ± 0.16	0.90 ± 0.18	0.87 ± 0.12
Incorrect response latency	Nicotine	2.96 ± 0.51	2.12 ± 0.36	1.91 ± 0.36	2.76 ± 0.53	2.48 ± 0.55	2.38 ± 0.46	2.37 ± 0.49
	Saline	2.49 ± 0.55	1.85 ± 0.42	1.32 ± 0.21	2.10 ± 0.55	2.12 ± 0.23	1.68 ± 0.29	2.59 ± 0.50
Go magazine latency	Nicotine	0.31 ± 0.03	0.34 ± 0.03	0.34 ± 0.04	0.33 ± 0.03	0.32 ± 0.03	0.33 ± 0.03	0.34 ± 0.04
	Saline	0.39 ± 0.07	0.35 ± 0.08	0.31 ± 0.06	0.35 ± 0.08	0.33 ± 0.08	0.36 ± 0.09	0.34 ± 0.05
No-go magazine latency	Nicotine	0.39 ± 0.04	0.41 ± 0.04	0.43 ± 0.04	0.42 ± 0.03	0.42 ± 0.06	0.38 ± 0.04	0.36 ± 0.05
	Saline	0.48 ± 0.06	0.48 ± 0.05	0.52 ± 0.06	0.47 ± 0.05	0.47 ± 0.06	0.55 ± 0.10	0.58 ± 0.06

Table 5.8: Each value represents the mean latency (seconds) ± SEM.

5.3: EXPERIMENT 4: EXAMINATION OF THE EFFECTS OF ACUTE NICOTINE CHALLENGES ON BEHAVIOURAL DISINHIBITION FOLLOWING PREVIOUS EXPOSURE TO NICOTINE

5.3.1. METHOD

5.3.1.1. Subjects

Subjects were 19 adult male Lister Hooded rats (Charles River, UK). All animals had completed Experiment 3 and had been previously chronically treated with either nicotine ($n = 10$) or saline ($n = 9$). Subject details have been previously outlined in the methodology of Experiment 3, section 5.5.1.1. At the start of testing, animals weighed approximately 370–400g.

5.3.1.2. Apparatus

See section 5.2.1.2.

5.3.1.3. Behavioural Testing

The behavioural procedure of the symmetrically reinforced go/no-go conditional visual discrimination task has been described previously in the general methodology of Chapter 3, section 3.2.1.3. The behavioural measures assessed in the paradigm are in addition detailed in Chapter 2. Details of subject training have been outlined previously in Experiment 3, section 5.2.1.3.

5.3.1.4. Drugs

Nicotine hydrogen tartrate salt, was dissolved in 0.9% saline and the pH adjusted to approximately 6, using 0.1M sodium hydroxide. Nicotine (0, 0.125, 0.25 and 0.5mg/kg) was administered s.c. in a volume of 1ml/kg to assess the effects of acute nicotine challenges on behavioural disinhibition following the chronic administration of drug treatment. All doses were calculated as free base and prepared freshly on each test day. Nicotine was administered 10 minutes prior to the operant test session.

5.3.1.5. Design and Procedure

Assessment began three weeks following the initiation of spontaneous withdrawal. All animals had returned to baseline levels of accuracy of performance observed prior to the initiation of chronic drug treatment. Furthermore no differences between groups in terms of their accuracy on the task were present.

The effects of acute nicotine challenges were examined using a mixed design, with treatment group (either previously chronically treated with saline or nicotine) as the between subject factor and acute drug treatment condition as the within subject factor. Treatment conditions were administered according to a Latin square design with a minimum of 72 hours between

drug administrations. It was essential that animals had returned to baseline performance prior to the subsequent treatment condition being administered. Baseline performance was defined as behaviour in the task deviating no greater than 5% from the accuracy of performance reached by the subject prior to the initiation of acute drug challenge regime. In the week prior to the commencement of acute nicotine testing, animals were habituated to injection procedures on two occasions, with subjects s.c. injected with 1ml/kg saline. Experimentation followed standard operant testing procedures, which have been outlined previously in the general methodology of Chapter 2, section 2.5.2. All operant testing took place during the light phase of their LD cycle between 1000h and 1400h.

5.3.1.6. Statistical Analysis

To ensure animals had both returned to baseline levels of performance and that no differences were present between treatment groups, accuracy of performance during the first three days following the third week of withdrawal were compared to the average baseline accuracy observed in the week prior to the initiation of chronic drug treatment. Data was analysed by a two-way mixed ANOVA with test session as the within subject factor and treatment group as the between subject factor.

Across all behavioural parameters assessment of the effects of acute nicotine following chronic nicotine exposure was conducted by a two-way mixed ANOVA, with treatment dose as the within subject factor and treatment group as the between subject factor. Significant main effects were assessed further by Bonferroni post hoc comparisons. Significant treatment dose x group interactions were explored further by one-way repeated measures ANOVAs examining the nicotine dose-response by group, in addition to independent t-tests comparing groups at individual treatment doses (Bonferroni correction of $p < 0.0125$). Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction. The homogeneity of variance of between subject variables was assessed by Levine's test. All data prior to analysis was assessed for normality and transformed where necessary (see also section 2.7). If data could not be successfully transformed, then the non-parametric Friedman test was employed to examine, by treatment group, the dose-response to nicotine, followed where appropriate by Wilcoxon procedures. Mann Whitney U tests were applied to the data to allow group comparisons at each treatment dose. In all cases of analysis α values of $p < 0.05$ were deemed statistically significant.

5.3.2. RESULTS

Magazine omissions (the failure to collect reward) rarely occurred throughout drug experimentation and therefore analysis of this behaviour was not conducted.

5.3.2.1. Return to Baseline

Statistical comparisons of accuracy of responding in the task to that of performance prior to the initiation of chronic drug treatment revealed that overall accuracy and independent performance during Go and No-go trials had returned to baseline levels, as indicated by the lack of significant main effects of test session ($df = 3,51$, all $F \geq 0.105$, N.S.). Furthermore, treatment groups did not differ in their accuracy across measure as shown by the non-significant main effects of group ($df = 1,17$, all $F \geq 0.001$, N.S.) and group x test session interaction found ($df = 3,51$, all $F \geq 1.377$, N.S.).

5.3.2.2. Accuracy of Performance

Acute nicotine reduced the total percentage of correct trials performed on the task in a dose related manner ($F(3,51) = 28.174$, $p < 0.001$). Post hoc analysis revealed that all doses of nicotine significantly reduced accuracy of overall performance in comparison to saline control treatment, with the greatest effect shown at the highest 0.5mg/kg dose (all $p < 0.001$). Furthermore, the highest dose significantly reduced accuracy in comparison to the lower 0.125mg/kg and 0.25mg/kg nicotine doses (all $p < 0.05$) (see Fig.5.6 (a)). Although no main effect of group was found ($F(1,17) = 2.944$, $p = 0.104$) a significant dose x treatment group interaction was revealed ($F(3,51) = 3.592$, $p = 0.020$). Further analysis demonstrated that whilst acute nicotine treatment significantly affected both previously treated saline ($F(3,24) = 7.125$, $p = 0.001$) and nicotine treated animals ($F(3,27) = 29.213$, $p < 0.001$), those pre-exposed to chronic nicotine were more sensitive to the effects, with a significant reduction in accuracy shown across all doses tested in comparison to the control dose (all $p < 0.05$) (see Fig. 5.6 (a)). Furthermore, following the highest treatment dose overall accuracy was significantly reduced in comparison to the 0.125mg/kg nicotine dose ($p < 0.05$). In contrast, in nicotine naïve animals nicotine reduced the total percentage of correct trials only at the highest dose in comparison to control ($p < 0.05$). Between group comparisons at each dose demonstrated that animals previously treated with nicotine performed significantly poorer than animals previously treated with saline following the 0.25mg/kg nicotine ($t = 1.104$, $df = 17$, $p = 0.012$).

Analysis of performance during Go trials revealed a significant main effect of nicotine on the ability to correctly respond during these trials ($F(3,51) = 3.281$, $p = 0.028$). Post hoc analysis however failed to locate significant differences between treatment doses tested (all $p > 0.05$). No significant between group effect was found ($F(1,17) = 1.503$, N.S.), however a significant dose x treatment group interaction was indicated ($F(3,51) = 3.418$, $p = 0.024$). Individual one way ANOVA of treatment groups revealed no significant main effect of treatment dose on the accuracy of performance during Go trials, for either previously treated saline ($F(3,27) = 1.379$, N.S.) or nicotine animals ($F(1.789, 14.313) = 3.804$, N.S) (see Fig. 5.6 (b)). Furthermore no significant differences between treatment groups were located across individual test doses (all t

$\leq 3.99, df = 17, N.S.$).

Analysis of No-go trials revealed a highly significant main effect of acute treatment found ($F(3,51) = 31.395, p < 0.001$). Post hoc analysis demonstrated that all doses of nicotine reduced accuracy of performance during No-go trials in comparison to saline control (all $p < 0.001$). The effect at the highest dose differed significantly from performance after the 0.125mg/kg treatment dose ($p < 0.05$). Although no significant main effect of treatment group was observed ($F(1,17) = 3.949, N.S.$), a significant group \times treatment dose interaction demonstrated group differences in response to acute nicotine treatment ($F(3,51) = 4.369, p = 0.008$). One way ANOVA demonstrated a significant main effect of treatment dose for both the saline ($F(3,24) = 7.141, p = 0.001$) and nicotine ($F(3,27) = 28.173, p < 0.001$) treatment groups. For the saline treatment group, performance was significantly reduced in comparison to saline control at the 0.5mg/kg ($p < 0.01$) dose. Conversely, a dose related reduction in accuracy of responding during No-go trials was observed in the nicotine treatment group. Performance during No-go trials was significantly impaired by all doses tested relative to saline control (all $p < 0.05$). Furthermore, the performance of animals in the nicotine treatment group was significantly worse following treatment of both 0.25mg/kg and 0.5mg/kg in comparison to the lowest, 0.125mg/kg, nicotine dose tested (all $p < 0.05$). Comparisons between groups across doses moreover revealed that nicotine group animals performed No-go trials significantly poorer than the saline treatment group at 0.25mg/kg dose ($t = 6.754, df = 17, p = 0.010$), whilst differences in performance at the 0.5mg/kg doses just missed significance ($t = 1.740, df = 17, p = 0.049$).

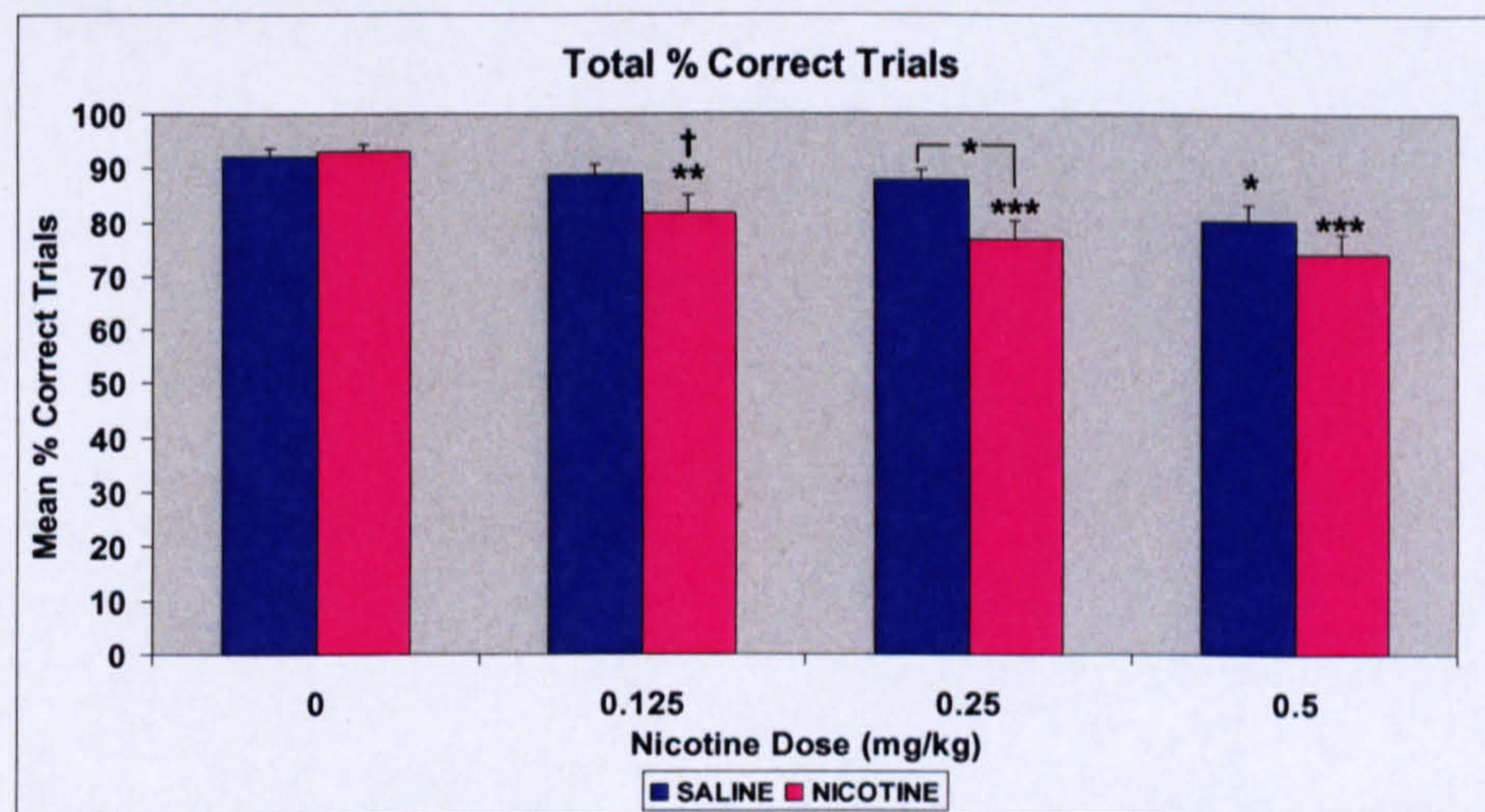


Fig. 5.6 (a)

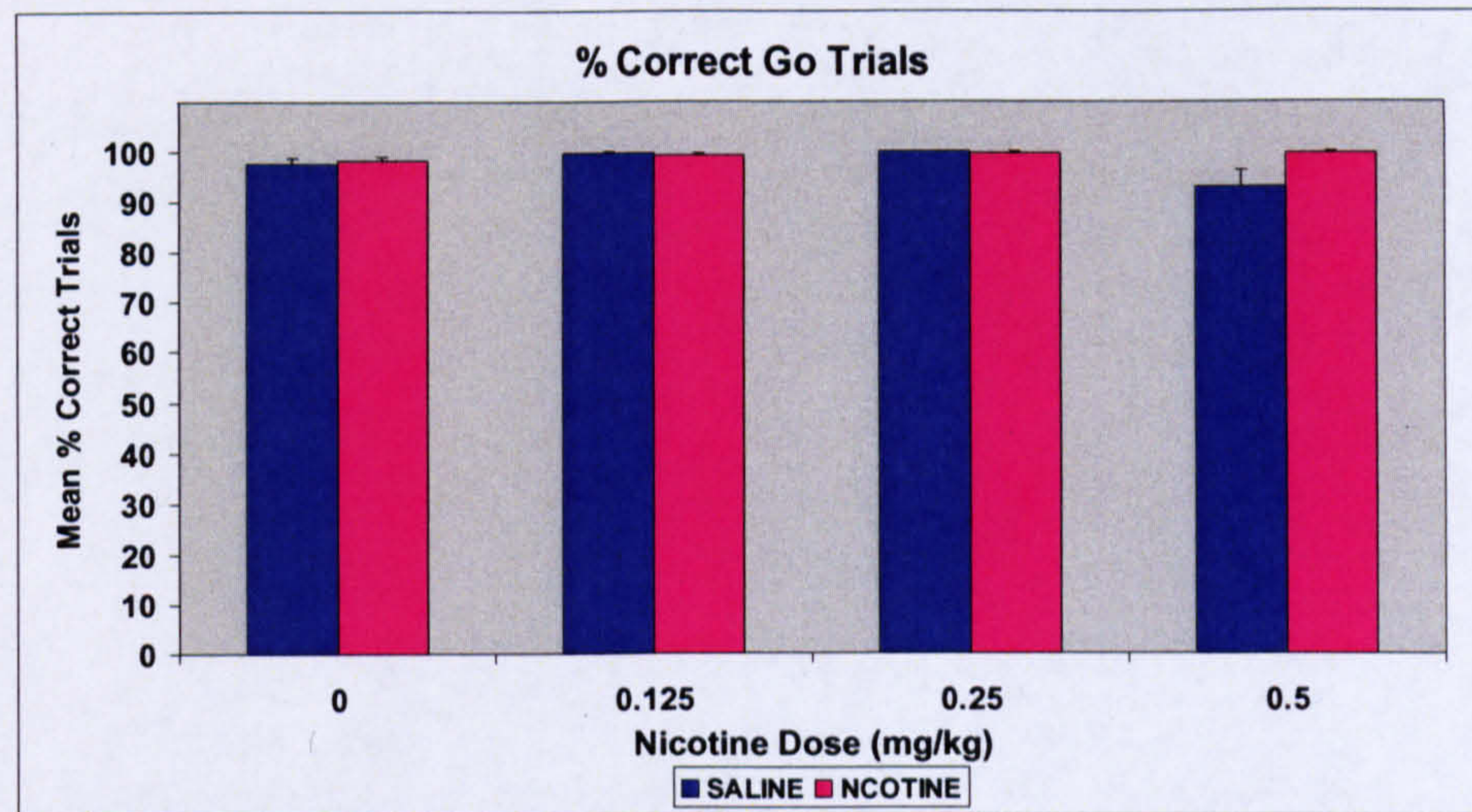


Fig. 5.6 (b)

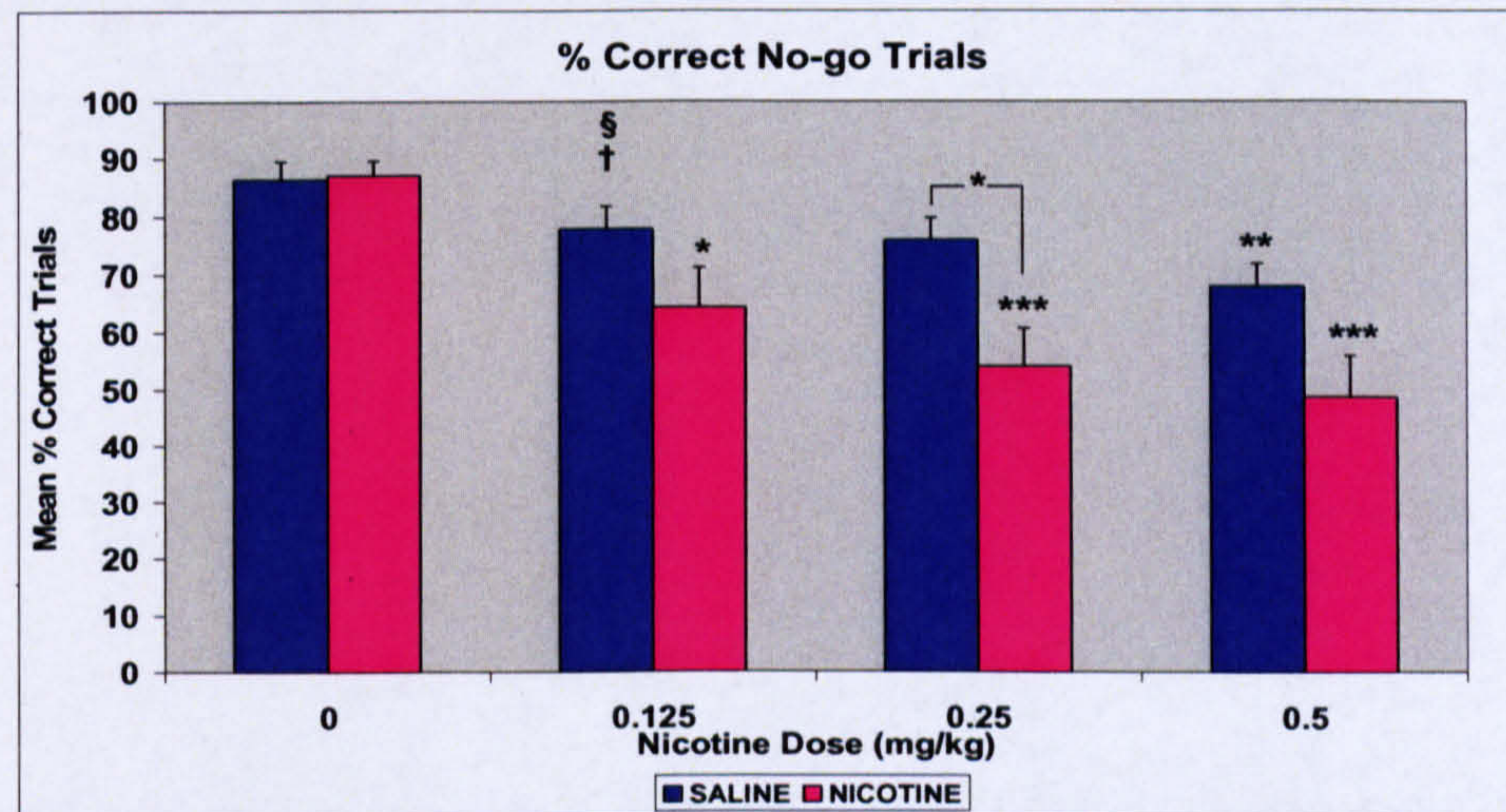


Fig. 5.6 (c)

Fig. 5.6 (a-c): Accuracy of Responding: the effects of acute nicotine administration on percentage correct Total trials (a), Go trials (b) and No-go trials (c). Each bar represents the mean percentage correct trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5 mg/kg §, $p < 0.05$, §§, $p < 0.01$, §§§, $p < 0.001$ (Bonferroni comparison) as compared to 0.25 mg/kg nicotine dose.

5.3.2.3. Anticipatory Responding

Figs 5.7 (a-d) illustrate the frequency of anticipatory responding across the dose range tested. Assessment of early responses demonstrated a significant main effect of dose for this anticipatory measure during both Go and No-go trials (all $F(3,51) \geq 2.704$, $p \leq 0.05$). During Go trials early responses increased significantly at the 0.125mg/kg dose in comparison to saline treatment ($p < 0.05$). Post hoc analysis of early responses during No-go trials however, indicated no significant differences between doses. No significant main effect of treatment group was observed on early responses during both Go and No-go trials (all $F(1,17) \leq 1.687$, N.S.), although dose \times treatment group interactions just failed to reach significance (all $F(3,51) \leq 2.464$, $p \geq 0.052$). As shown in Fig. 5.7 (a-b), in contrast to nicotine naïve animals those previously treated with nicotine displayed a trend of a greater increase in the frequency of early responding following acute treatment.

Acute nicotine failed to affect the frequency of inappropriate magazine entries during the stimulus presentation of both Go and No-go trials (all $F(3,51) \leq 1.104$, N.S.). This lack of effect was furthermore consistent across treatment groups, with both between group (all $F(1,17) \leq 0.935$, N.S.) and dose x group interactions failing to reach significance (all $F(3,51) \leq 0.669$, N.S.) (see Fig. 5.7 (c-d)).

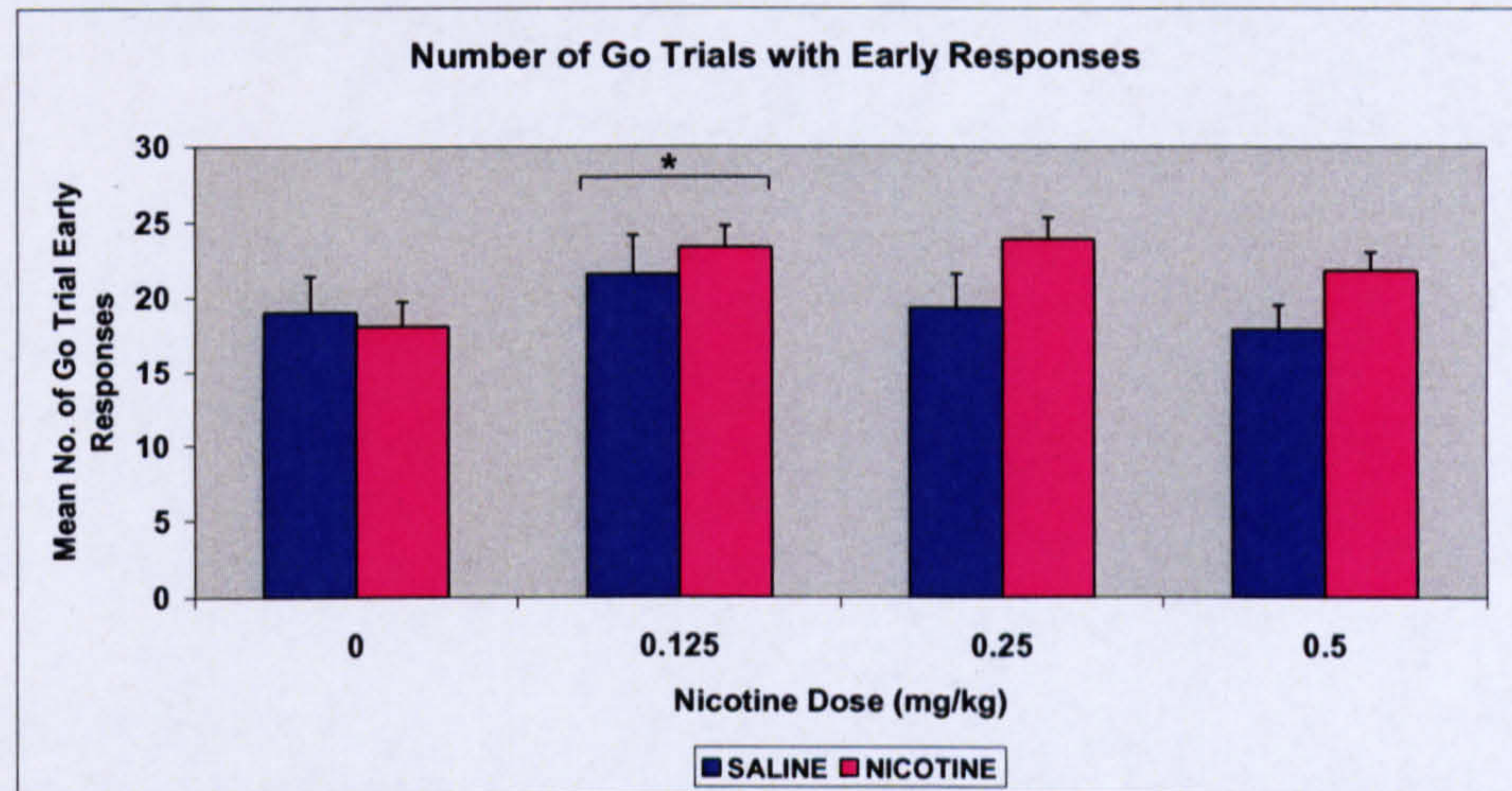


Fig. 5.7 (a)

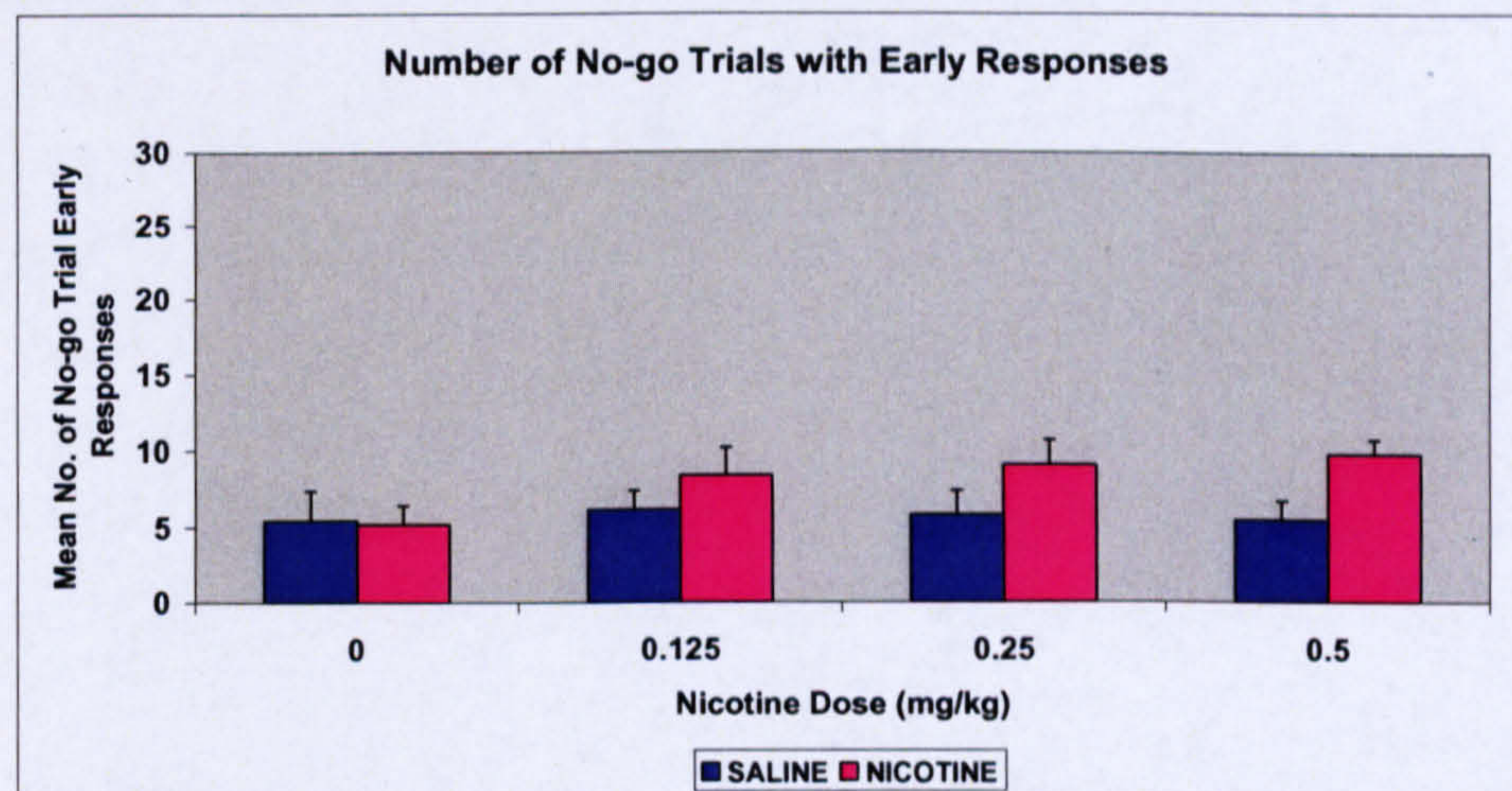


Fig. 5.7 (b)

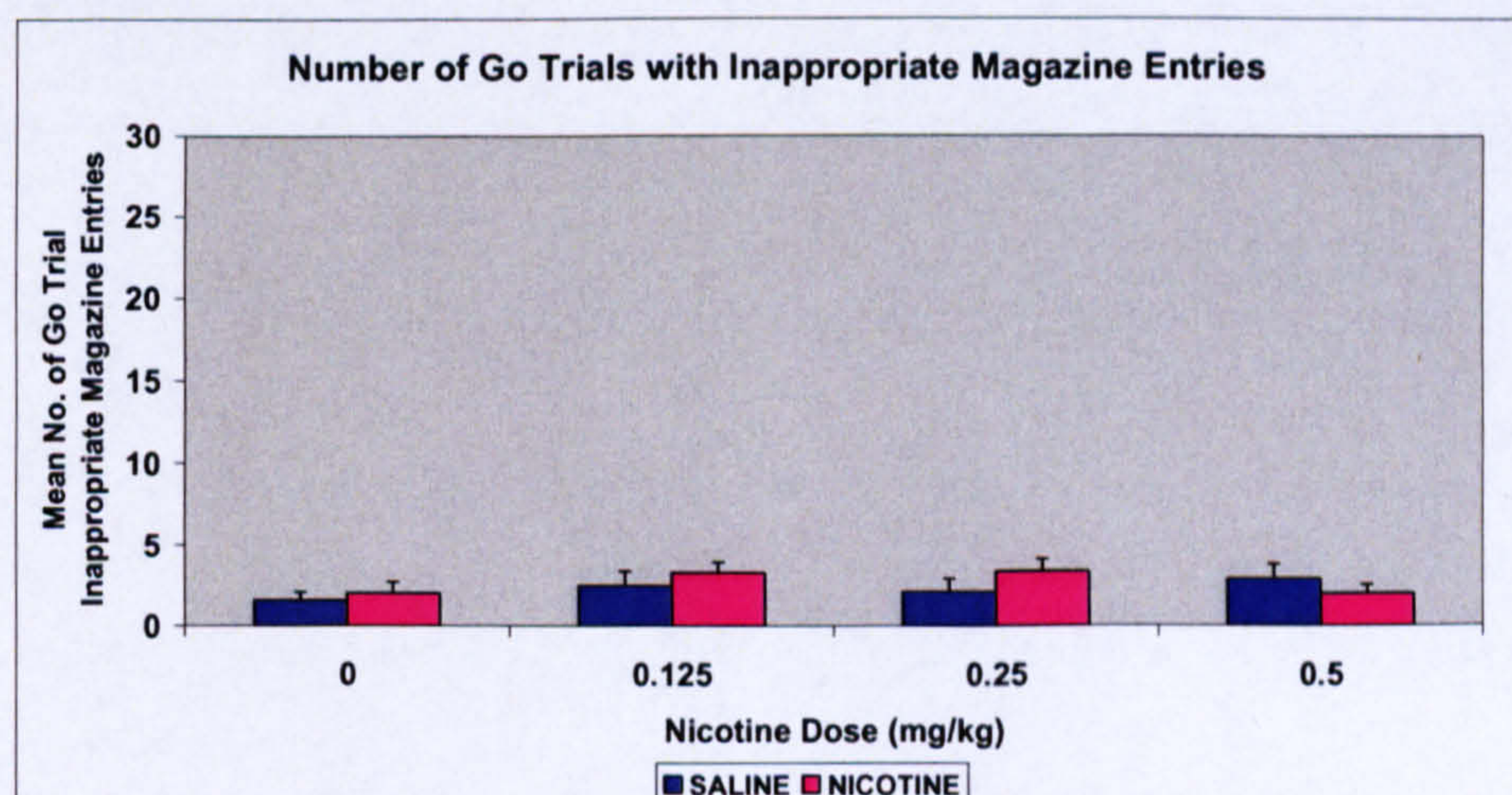


Fig. 5.7 (c)

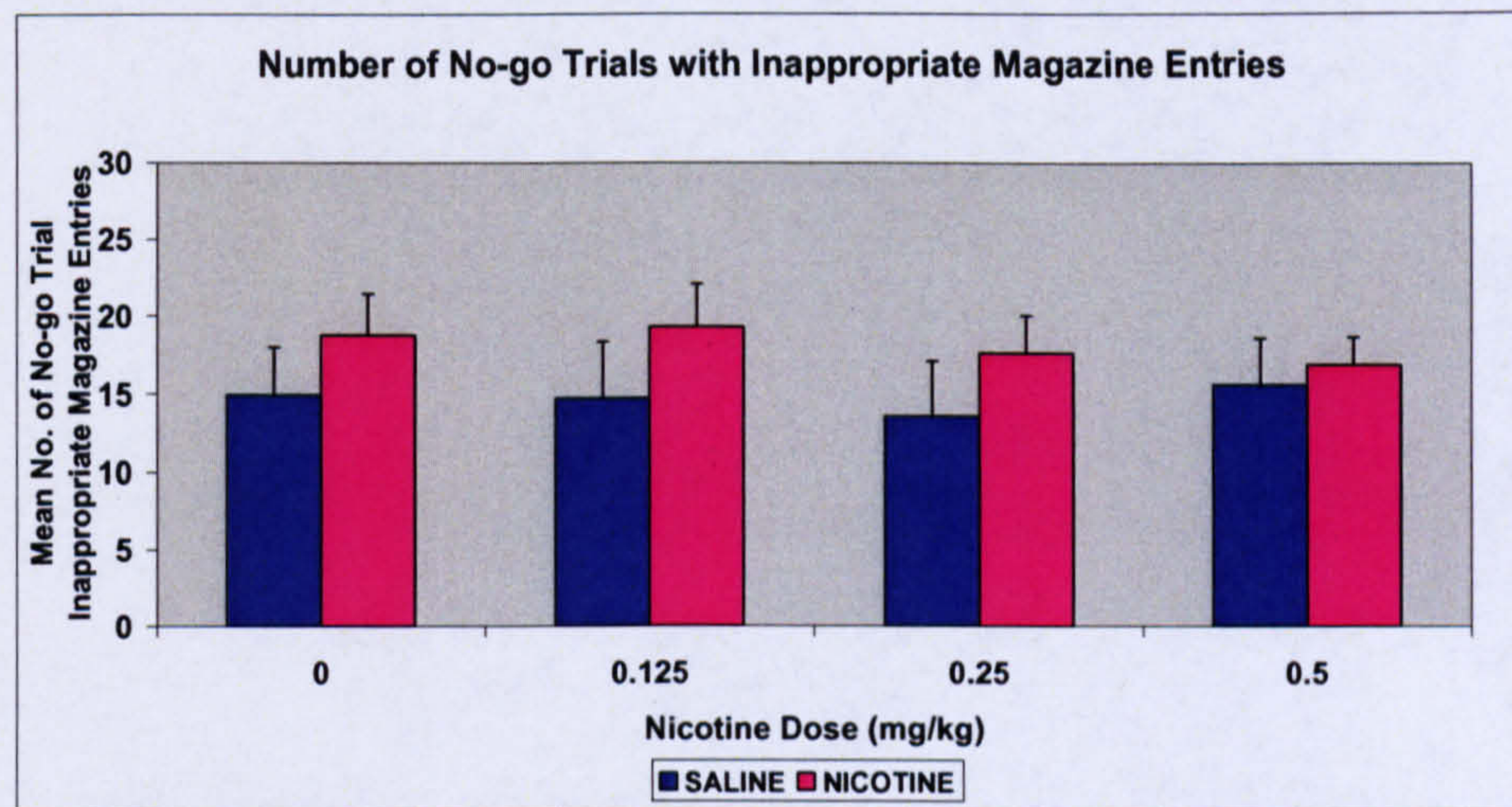
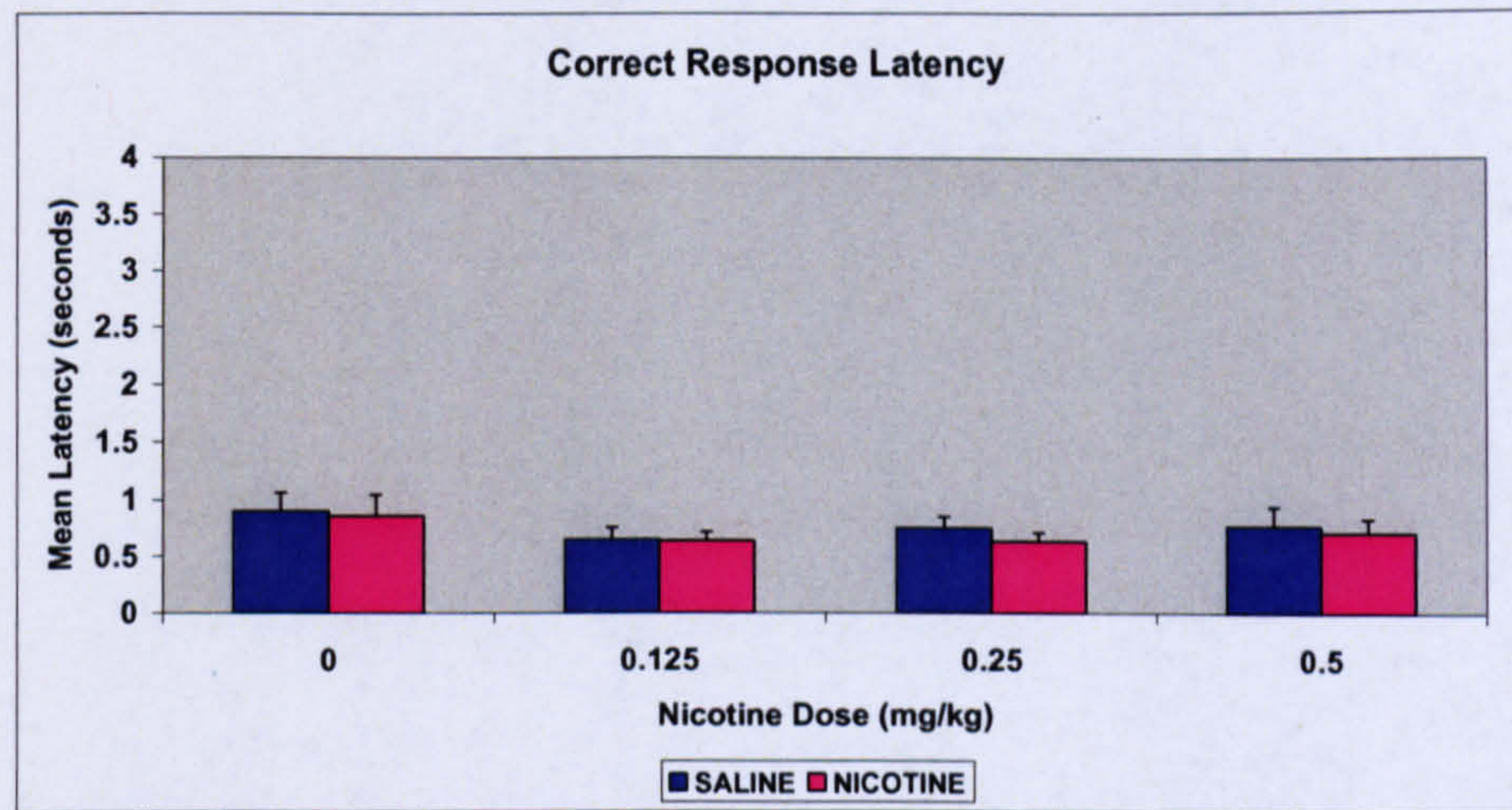
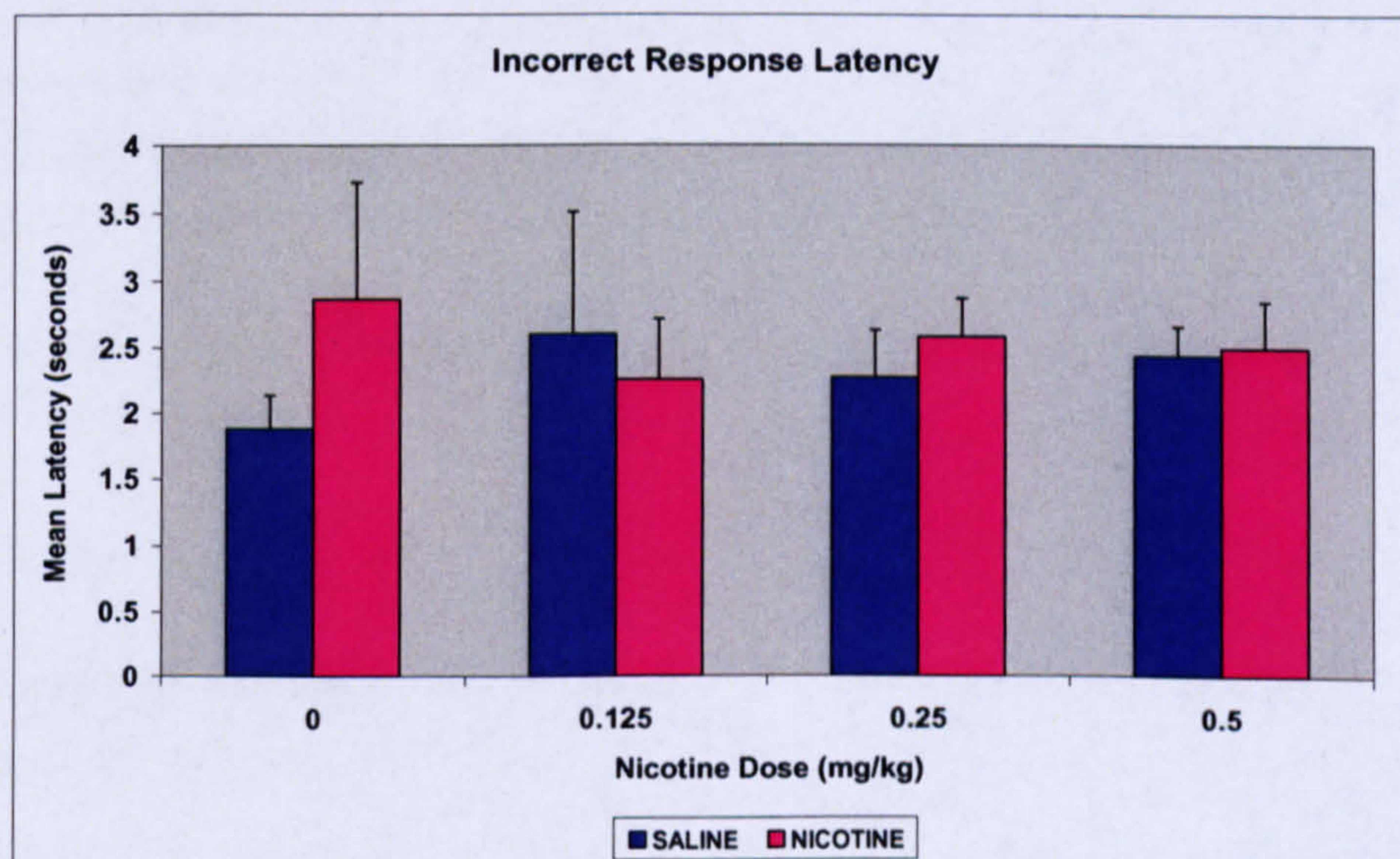
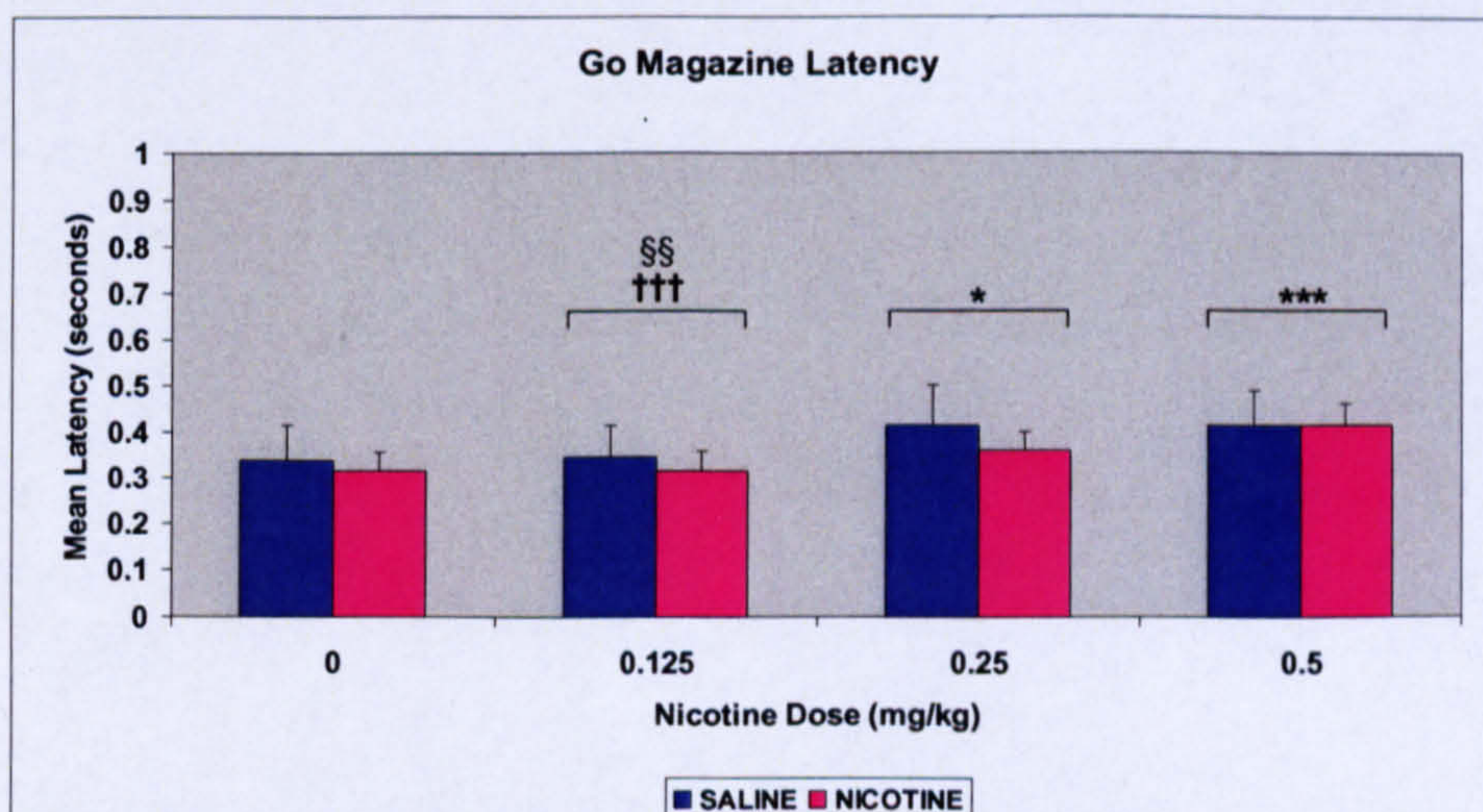
**Fig. 5.7 (d)**

Fig. 5.7 (a-d): Anticipatory Responding: the effects of acute nicotine on early responses during Go trials (a) and No-go trials (b). Anticipatory Responding: the effects of acute nicotine on inappropriate magazine entries during Go trials (c) and No-go trials (d). Each bar represents the mean number of trials \pm SE. * $p < 0.05$, (Bonferroni comparison) main effect of treatment dose, as compared to saline control.

5.3.2.4. Speed of Responding

Figs. 5.8 (a-d) illustrate the effect of acute nicotine challenges on measures of speed of responding. No significant main effect of dose was observed on correct or incorrect response latency (all $F(3,51) \leq 2.333$, N.S.). Response latency during both trials was furthermore similar across groups with a lack significant main effect of group (all $F(1,17) \leq 0.369$, N.S.) and dose \times group interactions found (all $F(3,51) \leq 0.317$, N.S.).

Acute nicotine significantly increased Go trial magazine latencies in a dose related manner ($F(3,51) = 16.110$, $p < 0.001$), reaching significance at 0.25 and 0.5mg/kg doses in comparison to the control treatment (all $p < 0.05$). The slower latencies to collect reward at these doses differed significantly from the lowest 0.125mg/kg nicotine dose (all $p < 0.001$). However, no evidence of differences between treatment groups in response to acute nicotine on this measure were demonstrated, with a non-significant between group and dose \times group interaction displayed ($F(1,17) = 0.001$, N.S.; $F(3,51) = 1.114$, N.S., respectively). Analysis of latencies to collect the reward during No-go trials additionally revealed a significant main effect of treatment dose ($F(3,51) = 4.806$, $p = 0.005$), although post hoc analysis revealed no significant differences between nicotine doses and saline treatment. Instead the main effect highlighted the significantly slower latency to collect reward during No-go trials at the highest 0.5mg/kg dose in comparison to the lowest 0.125mg/kg treatment dose ($p < 0.05$). Once more a lack of difference between treatment groups was shown in response to nicotine, as evident by the non-significant between group and dose \times group interaction found ($F(1,17) = 2.119$, N.S.; $F(3,51) = 1.583$, N.S., respectively).

**Fig. 5.8 (a)****Fig. 5.8 (b)****Fig. 5.8 (c)**

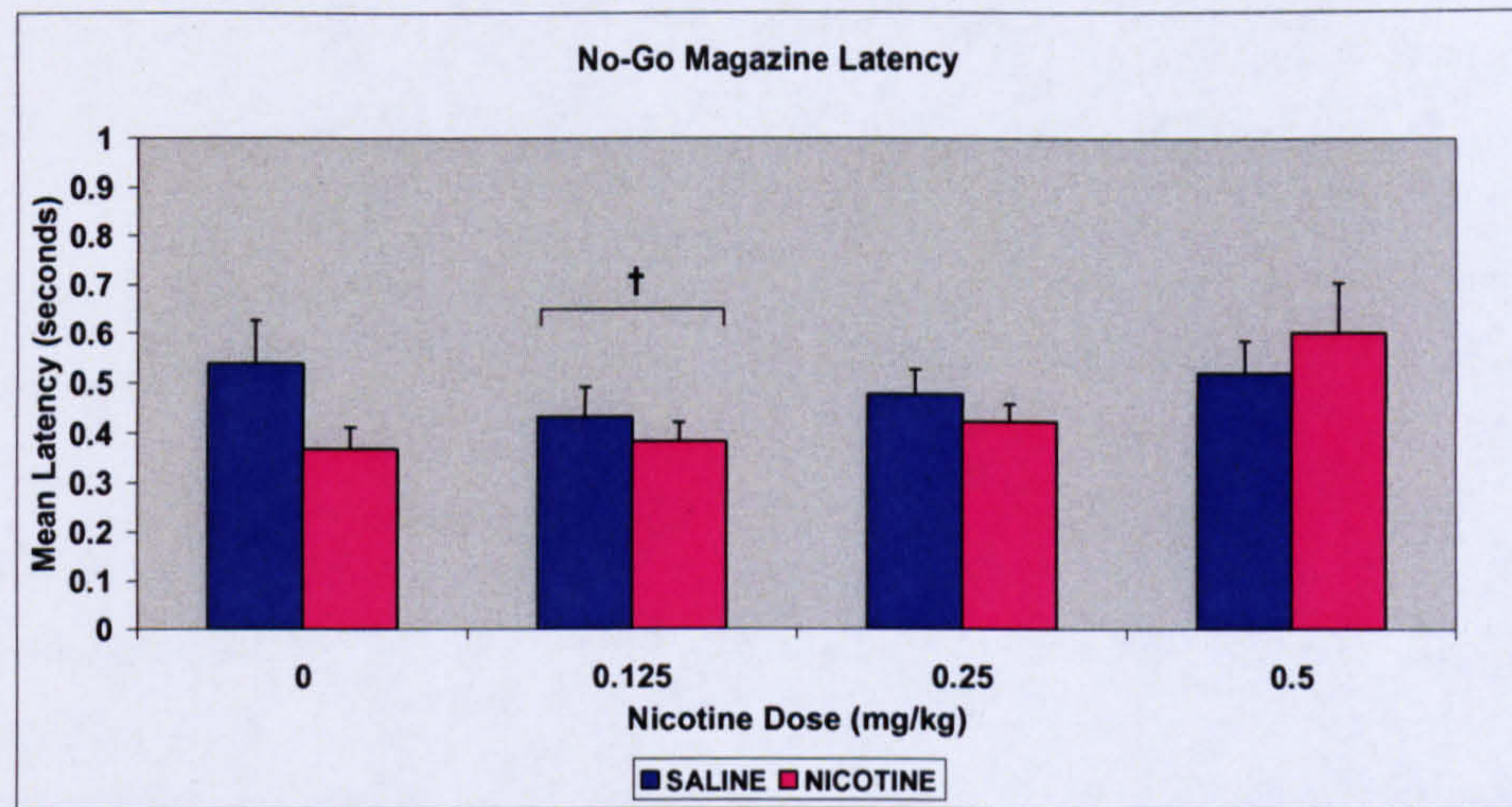


Fig. 5.8 (d)

Fig. 5.8 (a-d): Speed of Responding: the effects of acute nicotine administration on latency in seconds to respond correctly during Go trials (a) and incorrectly during No-go trials (b). **Speed of Responding:** the effects of acute nicotine administration on latency in seconds to collect reward following correct Go trials (c) and No-go Trials (d). Each point represents the mean latency in seconds \pm SE. $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) main effect of treatment dose, as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) main effect of treatment dose, as compared to highest 0.5 mg/kg §, $p < 0.05$, §§, $p < 0.01$, §§§, $p < 0.001$ (Bonferroni comparison) main effect of treatment dose, as compared to 0.25 mg/kg nicotine dose.

5.4. DISCUSSION

A strong association between nicotine dependence and behavioural disinhibition has been demonstrated in the literature (Spinella, 2002; Yakir et al., 2007); the causality of this relationship however remains to be fully determined. The studies of this chapter were the first of preclinical research to longitudinally examine the relationship between nicotine and behavioural disinhibition in a paradigm primarily adopted to assess inhibitory control, the symmetrically reinforced go/no-go task. The main findings were: (1) chronic nicotine treatment was associated with a substantial loss of inhibitory control, (2) cessation of chronic nicotine treatment led to a spontaneous nicotine abstinence syndrome during which a short lived rebound increase in inhibitory control was observed, (3) long term withdrawal was associated with heightened behavioural disinhibition and (4) despite nicotine induced effects on inhibitory control diminishing 21 days following termination of treatment, subjects remained hypersensitive to nicotine's effects on impulsivity. Each of these findings will be discussed in detail in the following sections.

5.4.1. Chronic Effects of Nicotine on Behavioural Disinhibition

Investigation of the effects of chronic nicotine treatment in Experiment 3 demonstrated marked deficits in overall performance on the go/no-go task in comparison to saline treated animals. The impairments in accuracy were restricted to performance on No-go trials providing strong support that chronic exposure to nicotine led to a dysfunction in inhibitory control. The

behavioural profile of nicotine treated animals further differed from the control group in that an enhanced frequency of early responding during Go trials was additionally observed. Nicotine treated animals also responded correctly during Go trials significantly faster than control subjects. Taken together, the observed behavioural changes during the chronic exposure to nicotine are indicative of reduced inhibitory control (Fletcher, 1993; Harrison et al., 1999).

The impulsive profile observed is not only in agreement with the effects of repeated nicotine treatment on premature responding and response latencies in the 5CSRTT (Blondel et al., 2000), but also supports the increase in behavioural disinhibition following chronic treatment of other psychostimulants such as cocaine (Jentsh et al., 2002). However, in contradiction to the findings of Blondel and colleagues, the effects of repeated nicotine treatment on inhibitory control did not increase with repeated administration. Conversely, the greatest impact of treatment was demonstrated during the first three days of chronic nicotine treatment (see Fig. 5.1 (a) and (c)). Although a significant treatment x day interaction was not observed, clearly present was a transient strengthening of inhibitory control during No-go trials during the final days of treatment, suggesting that the initial effect of nicotine on impulsivity recovered over testing. This pattern of effect is most likely due to the development of tolerance to nicotine. If tolerance does explain the observed transient effect of nicotine on impulsivity then animals only examined at the end of a comparable drug regime should display no differences in impulsive responding. Indeed adopting the precise nicotine dosing regime as in the present study, Shoaib and Bizarro (2005) reported no differences in anticipatory responding in the 5CSRTT between saline and nicotine treated animals on the final 7th day of osmotic pump implantation.

The elicitation of tolerance to nicotine actions during chronic or repeated drug exposure is not an uncommon finding, and has been observed across a range of behaviours in both laboratory animals (Stolerman, 1999; DiChiara, 2000) and human smokers (Perkins, 2002). These can include tolerance to nicotine's effects on nausea (e.g. Pomerleau 1995), locomotor depression (Clarke and Kumar, 1983), analgesia (Mousa, Aloyo, and Van Loon, 1988; Cepeda-Benito et al., 2006), brain reward threshold (e.g. Harrison et al., 2001) and drug discrimination (e.g. James et al., 1994; Robinson et al., 2007). Nicotine tolerance has been attributed to the nAChR desensitisation or inactivation observed during chronic exposure to the drug (Wonnacott, 1990; Marks et al., 1992; Littleton, 2001). Initially nicotine stimulates the nAChR leading to its activation but it is then followed by a rapid desensitisation during which the inactivated receptor fails to respond nicotine (Corringer et al., 1998; Quick and Lester, 2002). The period of inactivation becomes greater with longer term drug exposure (Mansvelder, Keath and McGhee, 2002) leading to the up-regulation of nAChRs and basis for nicotine tolerance (Collins et al., 1990; Wonnacott, 1990; Littleton, 2001). Interestingly, the subunits that have been found to desensitise most rapidly are the $\alpha 4\beta 2$ and $\alpha 7$ receptors, subunits which also display the longest

duration of inactivation and greatest up-regulation during chronic nicotine exposure (Olale et al., 1997; Quick and Lester, 2002; Nguyen, Ramussen and Perry, 2003). As discussed in Chapter 4, both these nicotinic receptor subunits have been implicated in the mediation of nicotine-induced disinhibition, as measured by the 5CSRTT (Blondel et al., 2000; Grottick and Higgins, 2000; Keller et al., 2005; Hoyle et al., 2006). As clear demonstration has been made that nicotine mediates its effects on disinhibition in the go/no-go task via central nAChR (see Chapter 4), it is therefore possible that the up-regulation of these receptors may underlie the developed tolerance to nicotine's initial impact on impulsivity in the present study.

Why therefore did Blondel and et al. (2000) demonstrate a contrasting sensitisation to the effects of nicotine on impulsive responding? The most likely explanation for the discrepancy lies in the varying patterns of nicotine dosing adopted across studies. Tolerance to nicotine's effects is most readily observed when the drug is administered repeatedly in close succession, a drug regime that was conducted in the present study and that is more comparable to the pattern of drug use shown by dependent smokers (e.g. Post, 1980; Benwell, Balfour and Birrek, 1995). In contrast, sensitisation to nicotine is more likely to manifest following intermittent dosing (DiFranza and Wellman, 2007), a pattern of nicotine dosing adopted by Blondel et al., (2000).

As discussed previously in Chapter 4 (section 4.4), nicotine induced disinhibition cannot be easily accounted for by alternative explanations such as impairments in conditional discrimination, working memory, attention and timing ability (e.g. Terry et al., 1996; Levin et al., 1998; Mirza and Stoleran, 1998; Decamp and Schneider, 2006; Rusted and Trawley, 2006; Spinelli et al., 2006; Day et al., 2007). Furthermore, increased intolerance to delayed reward is unlikely to account for the observed inability to withhold responding during No-go trials, as a reduction in incorrect response latency was not shown during the chronic treatment of nicotine (Harrison et al., 1999).

As with the effects of acute nicotine on performance in the task, the impulsive responding observed is also unlikely to have been mediated by nicotine's anorectic effects (McNair and Bryson, 1983; Grunberg et al., 1986; Levin et al., 1987; Blaha et al., 1998; Miyata et al., 1999; Zhang et al., 2001). Firstly, if nicotine's anorectic effects affected performance in the task then it would be expected that latency to collect reward in the task would increase (Bolles, 1965), this was not the case. Secondly, reducing motivation for reward in the preliminary research of Chapter 3 had no effects on task behaviour.

The loss of inhibitory control during chronic drug treatment could have arisen as a secondary effect of nicotine's ability to induce hyperactivity following repeated drug exposure (e.g. Clarke and Kumar, 1983). Although the decrease in response latency that was observed during

treatment could be indicative of hyperactivity, the effect was restricted to Go trial response latencies. If indeed the observed loss of inhibitory control was a consequence of nicotine increased motor activity, then rather than being explicitly associated to the correct responding during Go trials it would be expected that a reduction in latency would extend to all speed of responding parameters in the task.

It therefore appears, that most likely, chronic nicotine led to a genuine impairment in inhibitory control and suggests that the heightened impulsivity observed in smokers is a consequence of chronic nicotine use (Spinella, 2002; Yakir et al., 2007). Based on the present findings smokers may however become rapidly tolerant to the nicotine-induced effects on impulsivity, possibly accounting for the lack of reported effects of acute nicotine on behavioural disinhibition in current smokers (Bekker et al., 2005a). As the greatest effects of nicotine on disinhibition appear to be during the initial period of drug treatment it may be the case that a loss of inhibitory control may be a key component during the early stages of addiction (Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004). Nicotine intake during this stage may lead to the loss of control over drug seeking and taking behaviour leading to the continued maintenance of drug use.

The potential neurobiological mechanisms that may be mediating the effects of nicotine on impulsivity have been proposed in detail previously in Chapter 4. Most likely to be involved is the stimulated release of DA and 5-HT in regions associated with inhibitory control, including the VTA and its associated projections (Di Chiara and Imperato, 1988; Benwell and Balfour, 1992; Ribeiro et al., 1993; Nisell et al., 1996; Harrison et al., 1999; van Gaalen et al., 2006; Pattij et al., 2007; Picton et al., 2007). However, it is important to consider that chronic nicotine exposure has been associated with a decline of DA release within the regions of the VTA and the NAc shell (Ramussen and Czachura, 1995; Cardoni and Di Chiara, 2000), believed in part to be mediated by the inactivation and up-regulation of nicotinic receptors (Watkins, Koob and Markou, 2000a). Evidence suggests that DA receptor stimulation is crucial for the effects of nicotine induced disinhibition (van Gaalen et al., 2006); therefore it is possible that the observed tolerance to nicotine's effects may have been the result of the desensitisation of receptors at the molecular level and resultant decline in synaptic DA release.

5.4.2. Effects of Initial Nicotine Withdrawal on Behavioural Disinhibition

The cessation of chronic nicotine treatment gave rise to spontaneous nicotine withdrawal, supported by the significant elevation of somatic signs. Overall somatic symptoms peaked at 24 hours post pump removal and remained significantly elevated (with the exception of 60.5), for 84.5 hours following termination of treatment (see Fig. 5.4). Teeth chattering and chews were the earliest symptom to manifest, arising significantly in comparison to the control group as

early as 6 hours following cessation of treatment. The most frequently observed signs were scratches and foot licks, incorporated here within the miscellaneous category. During this period chronically saline treated animals, in contrast, demonstrated no evidence of an elevation of somatic signs. Consistent with previous research, the results therefore confirm that a nicotine abstinence syndrome is precipitated following the termination of chronic nicotine treatment (Malin et al., 1992; Hildebrand et al., 1997; 1999; Epping-Jordan et al, 1998; Watkins et al., 2000a; Harrison et al., 2001). Based upon aggregated behavioural signs the time course of the withdrawal syndrome was comparable to that of Harrison et al. (2001), however was longer than that reported by other previous research despite comparable nicotine regimen across studies (Malin et al., 1992; Hildebrand et al., 1997; 1999; Epping-Jordan et al, 1998). Furthermore, whilst the withdrawal syndrome in the current study was characterised most greatly by a significant increase in scratches and foot licks, the prominent somatic symptoms reported previously were conversely gasps, writhes and ptosis (Hildebrand et al., 1997; 1999; Epping-Jordan et al, 1998; Malin et al., 1992). This discrepancy could possibly be due to differences in rat strain adopted across studies, which may give rise to varying somatic symptomatology.

During the early stages of nicotine deprivation overall performance in the task was significantly *higher* than that of the saline treatment group. This was attributed to a greater ability to withhold responding during No-go trials whilst active responding during Go trials remained comparable to control subjects. These findings indicate that in direct contrast to chronic drug effects, early stages of nicotine withdrawal is associated with an increase in inhibitory control, an effect which is at its maximum at 12 and 60 hours post cessation of drug administration. Nicotine withdrawal had no significant effect on anticipatory measures or speed of responding in the task. Although evidence of an increase in the latency to collect the reward during Go trials was observed, statistical analysis failed to locate significant differences between groups across test sessions. These findings are in agreement with recent reports of the effects of both spontaneous and DH β E precipitated nicotine withdrawal in nicotine dependent animals in the 5CSRTT (Shoiab and Bizarro, 2005). Adopting a comparable drug regime as in the present study, spontaneous and precipitated nicotine withdrawal led to a significant reduction in anticipatory impulsive responding in comparison to saline treated animals, the effect however was more pronounced following DH β E precipitated withdrawal (Shoiab and Bizarro, 2005).

The findings of the present research however appear to be in direct opposition to the reported heightened impulsive responding on the CPT in smokers following both 24 hour (Hatsukami et al., 1989) and overnight abstinence (Dawkins et al., 2007) relative to when nicotine satiated. The version of the CPT adopted in this research could however be argued to be a more cognitively demanding paradigm than the present go/no-go task, possibly accounting for the discrepancy in findings. For example the CPT adopted by Dawkins et al., (2007) depended

heavily on attention and working memory, requiring participants to attend to the presentation of a series of five-digit sequences and to respond when a sequence was identical to the former. Smoking abstinence is associated with deficits in cognitive processing including both attention and working memory (e.g. Al-Adawi and Powell, 1997; Morris 1999; Mendrek et al., 2006). Impairments can arise as early 4h following cessation and peak at 12-48 hours of withdrawal (Snyder et al., 1989), making it possible that the impaired inhibitory control on the CPT may have been a secondary effect of nicotine deprivation on cognitive processing within the task.

The contrasting increase in inhibitory control relative to the reduced levels observed during chronic nicotine treatment is however consistent with the rebound syndrome of nicotine withdrawal, which is characterised by both behavioural and affective symptoms that are often in direct contrast to the effects of chronic exposure (Watkins et al., 2000b; Koob and Le Moal, 2001; Hughes, 2007a). For example nicotine produces pleasurable reinforcing effects, a reduction in anxiety, decrease in appetite and positive effects on cognition (e.g. Pomerleau and Pomerleau, 1992; Stolerman and Jarvis, 1995; Heishman, 2002) Nicotine withdrawal in contrast can be associated with depressed mood, anxiety, increased appetite and deficits in cognition (Hughes et al., 1991; Heishman, 2002; Hughes, 2007b). The present research findings suggest that the characteristic rebound syndrome of nicotine withdrawal may also extend to incorporate nicotine's effects on inhibitory control. When considering the possible processes that may govern this short lived decrease in impulsivity, it is unlikely that an increase in appetite following termination of nicotine is mediating the effect (Grunberg et al., 1986; 1987; Miyata et al., 1999). Experiment 1C revealed no effects on accuracy of responding in the task following an increase in primary motivation (Bizarro and Stolerman, 2003). Furthermore, no evidence of a decrease in response latency or latency to collect food was observed; conversely indication of a slowing in collection of reward was shown during Go trials.

It is further unlikely that deficits in attention during nicotine withdrawal, an effect replicated in the animal literature, can account for the findings (Shoiab and Bizarro, 2005). If indeed attentional processes do play an important role in accuracy of performance in the go/no-go task then it would be predicted that a decrease in accuracy in the model should be displayed during drug deprivation. Animals experiencing nicotine withdrawal in the present research in contrast displayed an increase in accuracy of responding in the task.

An alternative process that may be mediating the increase in inhibitory control is related to the affective symptoms of nicotine withdrawal, in particular depression (Gilbert 1998; Baker et al., 2004). One of the key symptoms of depression is anhedonia, or "diminished sensitivity to reward" (DSM IV; Markou et al., 1998). Following the termination of identical chronic drug regimes that were adopted in the present research, changes in affective state have been reliably

shown in an animal model of withdrawal utilising intracranial self stimulation methodology (Epping-Jordan et al., 1998; Watkins et al., 2000b; Harrsion et al., 2001; Cryan et al., 2003). The manifestation of this symptom during withdrawal could have led to a reduction of the value of the food reward. This effect could have, in turn, decreased the motivation to earn the reward, thus increasing passivity in the task. Such passiveness in the current paradigm would favour performance during No-go trials, which requires animals to withhold responding. Based on the theory, it would also be predicted that diminished motivation for reward would additionally reduce active responding during Go trials and slow the speed of responding in the task. No differences in performance between treatment groups were however observed during Go trials, although it could be argued that with extensive testing active responding during these trials may have become a highly conditioned possibly habitual response and therefore less sensitive to changes in affective state. With the exception of latency to collect reward during Go trials, no evidence of alterations in speed of responding in the task were observed during nicotine deprivation. The increase magazine latency demonstrated during Go trials is an arguable indication of a reduction in motivation, however statistically the difference between treatment groups failed to reach significance. Although at this stage it cannot be dismissed that alterations in sensitivity to reward may play a role in mediating the improved accuracy during No-go trials, the lack of significant evidence of the influence of anhedonia on other variables within the task provides a strong argument against this possibility. Thus, it is highly likely that an improvement in inhibitory control, rather than diminished sensitivity to reward, is responsible for the greater ability to withhold responding on No-go trials during initial nicotine withdrawal.

Indication that early stages of nicotine deprivation are associated with a rebound increase in inhibitory control suggests that this subcomponent of impulsivity may not be involved in smoking relapse during the initial few days of nicotine withdrawal. Instead relief from aversive symptoms of withdrawal, which are at their greatest during the first week post termination of nicotine abuse, may play more of a crucial role in predicting relapse at this stage (Koob and LeMoal, 2001; Hughes 2007b).

Although the present study is unable to distinguish the neurobiological processes underlying the increase in inhibitory control during early withdrawal, understanding of the neurobiological theories of nicotine withdrawal may provide insight into the possible mechanisms involved. As previously discussed, at the molecular level one of the neural adaptations that take place during chronic nicotine exposure is the up-regulation of nicotinic receptors (Littleton et al., 2000; Kenny and Markou, 2001). When nicotine exposure ceases however these excess receptors remain unopposed and begin to recover from desensitisation; a process argued to perhaps mediate the symptoms of withdrawal (Watkins et al., 2000a; Kenny and Markou, 2001). The precise biological mechanisms of how this would occur still remain unclear, however strong

evidence has suggested that the downstream effect of nicotine abstinence is reduced DA release. Cessation of both the chronic treatment and self administration of nicotine in rodent models of withdrawal have been associated with a decrease in DA release in both the mesolimbic system and NAc, believed to mediate somatic and affective symptomatology respectively (Fung et al., 1996; Hildebrand et al., 1999; Watkins et al., 2000; Koob and Le Moal, 2001; Rahman et al., 2004). The findings that blockade of D1 and D2 receptors is capable of decreasing premature disinhibited responding in both the 5CSRTT (Harrison et al., 1997; Koskinen and Sirvio, 2000; Hahn et al., 2002; Shoaib and Bizarro, 2005; van Gaalen et al., 2006) and FCN (Liao and Cheng, 2000) provides potential evidence that alterations in this particular neurochemical system during withdrawal may modulate the increase in inhibitory control. This is further supported by the fact that the NAc has been implicated as an important brain structure that may modulate impulsive control (Reading and Dunnett, 1995; Christakou et al., 2004; Pothuizen et al., 2005). Interestingly, the most substantial decrease in DA neuronal firing in the VTA (which projects to the NAc) is suggested to appear in the first 2 days following cessation of nicotine treatment (Liu and Jin, 2004), a time period during which inhibitory control was at its greatest in the present research.

Whilst a reduction in DA release is one of the most dominant neurobiological theories of nicotine withdrawal, alterations in other systems may additionally play a role in the mediation of a decrease in impulsivity. For example, decreased serotonergic and glutamate functioning have additionally been implicated in nicotine withdrawal (Watkins et al. 2000a; Harrison et al., 2001; Kenny and Markou, 2004). Although it is currently unknown what role glutamate may play in modulating inhibitory control, as previously discussed extensive evidence has implicated serotonergic systems in the neurobiology of impulsive behaviour (Linnola, 1983; Soubrie, 1986; Harrison et al., 2001), suggesting strongly the potential involvement of this system in the observed alterations in impulsivity during the initial stages of drug deprivation.

5.4.3. Effects of Long Term Nicotine Withdrawal on Behavioural Disinhibition

By the second week of nicotine withdrawal the observed enhancement in inhibitory control had diminished and overall accuracy had returned to levels of that performed by the saline treatment group. Examination of independent performance during Go and No-go trials furthermore supported a lack of significant difference between treatment groups during this stage. However, as clearly shown in Fig.5.1 (c) from day 9 post termination of treatment, animals chronically treated with nicotine demonstrated an increasing deficit in accuracy of performance during no-Go trials. Whilst premature early responding did not differ between groups, inappropriate magazine entries were significantly enhanced during both Go and No-go trials in animals experiencing withdrawal. No group differences were indicated on speed of responding parameters during this stage of nicotine withdrawal.

During the third week of withdrawal overall accuracy of performance remained comparable across groups. This lack of difference between groups was mirrored by the performance during Go trials. In contrast however, the increasing deficit in performance during No-go trials that began in week two of withdrawal continued and reached statistical significance on day 17, whilst just missing significance on day 15 of withdrawal (see Fig. 5.1 (c)). As clearly shown in Fig. 5.1 (a-c), this deficit in inhibitory control recovered and the accuracy of responding in chronically nicotine treated animals were indistinguishable from the saline group by the 18th day post termination of treatment. No group differences were indicated during this stage on anticipatory or speed of responding measures in the task.

Taken together these findings indicate that longer term nicotine withdrawal is associated with an increasing deficit in inhibitory control, supported firstly by the enhanced anticipatory responding in week two of nicotine withdrawal and the inability to withhold responding during No-go trials at the early stages of week three of nicotine abstinence. Based on these findings it may be predicated that smoking relapse after cessation may be most prevalent during this time due to an enhanced inability to control drug seeking and taking behaviour. Interestingly, smoking relapse is often rapid with estimates suggesting that 75% of smokers relapse within the first two weeks of abstinence, a time period that according to the present research is associated with impairments in inhibitory control (Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995). With recent evidence additionally suggesting that greater levels of impulsivity can significantly predict relapse to smoking, these findings provide strong support that the heightened disinhibition observed may play a key role in mediating drug relapse during this stage of withdrawal (Doran et al., 2004; Krishnan-Sarin et al., 2007). Pharmacological and behavioural treatment interventions that target strengthening inhibitory control during this stage of abstinence may therefore prove to be an effective future intervention to aid smoking cessation.

Due to minimal research assessing the time course of chronic nicotine induced neuroadaptations during long term withdrawal, it is difficult to determine at this stage the neurobiological processes that may be modulating this heightened impulsivity. It has been demonstrated that following 12 days of repeated nicotine treatment VTA dopamine neuronal activity, although depressed during initial withdrawal, return to baseline levels by 10 days post termination of treatment suggesting that this particular neurobiological system may be playing a minimal role (Ramussen and Czachura, 1986; Liu and Yin, 2004). The continued dopaminergic activation of prefrontal pathways by chronic stimulants has however been argued to lead to longer lasting abnormalities in areas of the PFC, a brain region believed critically involved in the functioning of inhibitory control (e.g. Volkow et al., 1996; Goldstein and Volkow, 2002; Aron et al., 2004).

In human smokers recent research has demonstrated that chronic smoking, as with other drugs of abuse such as cocaine and methamphetamine, is associated with reduced gray matter volumes and densities in frontal brain areas including the ACC and occipital cortex relative to non-smokers (Brody et al., 2004). Long term abstinence is furthermore associated with significant hypoactivation, in the PFC in particular the orbitofrontal cortical areas (Neuhaus et al., 2006). If the neuroanatomical abnormalities within these regions do indeed reflect damage owing to long term exposure to nicotine, rather than some pre-existing condition, then this may indicate potential dysfunctional systems that may be modulating the heightened impulsivity during longer term withdrawal. In support of this theory only a brief exposure to nicotine has been shown to elicit neuritic damage in rodents (e.g. Xu et al., 2001; Abreu-Villaca et al., 2003).

Alterations in serotonergic activity associated with chronic nicotine exposure must also be considered as a possibly neurochemical modulator involved in the expression of disinhibited behaviour at this stage of withdrawal. Attenuated 5-HT levels have been demonstrated in regions of the limbic system (Olausson et al., 2001), hippocampus (Benwell and Balfour, 1982) and frontal cortex (Kirch et al., 1987) following repeated treatment with nicotine. The relationship between serotonergic functioning and impulsivity is complex. However, the inverse relationship between serotonergic functioning and impulsive behaviour is a neurobiological theory that has received support in models of inhibitory control, including the go/no-go task, thus making it a prime neurobiological candidate that may be mediating the relationship between long term nicotine withdrawal and impulsivity (Fletcher, 1993; Harrison et al., 1999).

As neurobiological processes were not assessed in the present research, the proposed neural adaptations that may underlie the observed disinhibited behaviour remain highly speculative. What the present research can elucidate however is that regardless of the neuroadaptations involved they appear to be a temporary effect, at least in terms of level of impulsivity expressed at baseline (see below). As clearly demonstrated performance across all parameters in the go/no-go task by 18 days post termination of treatment had returned to comparable levels of control group. Until now research has been unable to determine the relative permanence of any drug induced changes in impulsivity. Yakir et al. (2007) for example demonstrated that ex-smokers who had remained abstinent for a six month period displayed a level of inhibitory control on the CPT that was intermediate between current and never smokers. These findings could firstly suggest that the intermediary levels of inhibitory control in ex-smokers is a result of reversible nicotine induced changes on impulsive behaviour. At six months following termination of smoking however self control has yet to return fully to levels of never smokers. Conversely, the greater control over behaviour observed in ex-smokers could have been a trait that has enabled these individuals to successfully abstain. The present findings provide support for the former of these interpretations, with clear demonstration that nicotine induced increases in impulsivity

observed at baseline is a temporary effect.

5.4.4. Effects of Acute Nicotine Challenges on Behavioural Disinhibition in Animals Previously Exposed to Chronic Nicotine

Twenty one days following the termination of drug treatment the dose response to acute nicotine was compared across groups in order to determine whether changes in responsivity to nicotine's effects on impulsivity had developed following chronic treatment of the stimulant. Animals previously exposed to chronic nicotine displayed a significantly more profound impairment in overall accuracy of responding following acute nicotine administration in comparison to nicotine naïve animals. Animals chronically treated with nicotine demonstrated a significant reduction in accuracy of performance across all doses tested relative to the control dose. Conversely, nicotine naïve animals displayed a significant reduction only at the highest 0.5mg/kg dose tested in comparison to saline control. Furthermore, the chronic nicotine treatment group performed significantly poorer than the saline treatment group following acute treatment of the 0.25mg/kg dose. Independent analysis of Go trials revealed that performance during these trials remained unchanged following treatment. Although a significant group x dose interaction was indicated on this measure, further statistical analysis revealed that no significant differences between treatment groups were observed on accuracy of Go trial performance following acute drug treatment. The impaired overall accuracy of performance in the go/no-go task displayed by both treatment groups was instead clearly as a result of the inability to correctly withhold responding during No-go trials. Once again, the effect was more substantial in animals previously treated with nicotine. Acute nicotine dose dependently reduced accuracy during No-go trials in this group of animals. In nicotine naïve animals impaired performance was in contrast only observed at the highest 0.5mg/kg dose tested. Comparison of group performance at individual doses furthermore indicated that animals previously exposed to nicotine exhibited a greater inability to withhold responding following the 0.25mg/kg dose in comparison to nicotine naïve animals whilst differences in responding following the 0.5mg/kg dose just missed significance.

Previous nicotine exposure did not however differentially affect the response to acute nicotine on anticipatory responding measures in the task. The effect of acute nicotine challenges was similar in both groups. Early responding during Go trials was found to be significantly enhanced following acute treatment of 0.125mg/kg nicotine dose. Early responding during No-go trials and inappropriate magazine entries did not differ from that of anticipatory responding following control treatment. The effect of acute nicotine on speed responding was furthermore comparable across treatment groups. Both correct and incorrect response latencies remained unchanged following acute treatment. Conversely, latencies to collect the reward following Go trials significantly increased following treatment of 0.25 and 0.5mg/kg doses of nicotine. Although a

slowing in latency to collect reward was additionally observed during No-go trials, differences across doses only reached significance between the highest 0.5mg/kg dose and lowest 0.125mg/kg dose tested.

Taken together these findings demonstrate that both treatment groups demonstrated evidence of acute nicotine induced disinhibition. In nicotine naïve animals the effect on accuracy of responding was furthermore in a manner that directly replicated the findings of Experiment 2A. In contrast, in animals with previous nicotine exposure the observed nicotine induced loss of inhibitory control was a substantially more profound effect, providing strong evidence that chronic nicotine treatment can induce sensitisation to the effects of nicotine on behavioural disinhibition, an effect still present three weeks following the termination of drug treatment.

The present research is the first to establish that chronic nicotine can render subjects hypersensitive to nicotine's effects on behavioural disinhibition after period of sustained abstinence in a validated model of impulsivity. Research has previously demonstrated potential evidence of the sensitisation of the effects of nicotine on anticipatory responding in the 5CSRTT, with repeated nicotine administration leading to an increasing effect on impulsive responding (Grottick and Higgins, 2001; 2000; Day et al., 2007). A decrease in reaction time was however additionally observed in the majority of past research (e.g. Grottick and Higgins, 2000; 2001), making it difficult to dissociate the observed effects from that of nicotine induced hyperactivity that is additionally associated with repeated intermittent nicotine exposure (e.g. Clarke and Kumar, 1983).

Although behavioural sensitisation to nicotine can manifest as an increase in locomotor activity (Clarke and Kumar, 1983; Chaudhry, Turanis and Karler, 1988; Pierce and Kalivas, 1997), it is highly unlikely that the enhanced nicotine induced deficits in inhibitory control following chronic nicotine treatment in the present research are secondary to locomotor effects. A vital argument against this explanation is that acute nicotine in the task did not influence response latencies during both Go and No-go trials in either treatment group. Furthermore, the speed at which food rewards were collected displayed evidence of a *slowing* in latency, providing strong support for the notion that in the current study a dissociation can be made between nicotine sensitised disinhibition and psychostimulant induced hyperactivity.

Demonstration that latencies to collect reward during Go trials were significantly increased at the highest doses may be indicative of nicotine's established anorectic effects (McNair and Bryson, 1983; Grunberg et al., 1986; Levin et al., 1987; Blaha et al., 1998; Miyata et al., 1999; Zhang et al., 2001). It is unlikely however that alteration in primary motivation can account for the more substantial loss of inhibitory control displayed by animals with previous nicotine

exposure. Firstly, the increase in latency to collect reward following nicotine was an effect observed in both treatment groups. Furthermore, in Chapter 3 of this thesis decreasing motivation for reward through prefeeding was demonstrated to have minimal impact on inhibitory control in the go/no-go task. Although previous research has indicated potential evidence of an impact of pre-feeding on impulsive responding in paradigms such as the 5CSRTT, findings have demonstrated an effect opposite to that observed in the present study, where a decrease in impulsive responding was observed (Carli and Samanin, 1992; Harrison et al., 1997; Grottick and Higgins, 2000; Grottick and Higgins, 2002; Bizarro and Stoleran, 2003).

The increased magnitude of the effect of nicotine in animals with previous nicotine exposure appears therefore to reflect evidence of a genuine sensitisation to nicotine's effects on behavioural disinhibition. Behavioural sensitisation to drugs of abuse has long been argued by researchers to be greatly involved in drug seeking and taking behaviour (Robinson and Berridge, 1993). More specifically, the process is hypothesised to mediate the transformation for wanting the drug into craving for the drug. Traditionally behavioural sensitisation in animals has been argued to manifest as an increase in locomotor activity (Clarke and Kumar, 1983; Chaudhry, Turanis and Karler, 1988; Pierce and Kalivas, 1997). The present findings have however indicated that behavioural sensitisation to nicotine can additionally be reflected as enhanced behavioural disinhibition, supporting further the importance of a loss of control over behaviour as a key component of addiction (Jentsh and Taylor, 1999; Goldstein and Volkow, 2002; Luban et al., 2004).

According to the current study sensitisation to the effects of nicotine on inhibitory control were still present at 5 weeks following termination of treatment, a period likely to be equivalent to many years in a human smoker's lifespan. Whether effects can persist for a greater period is an avenue for future research, however it has been demonstrated that nicotine sensitised locomotor effects can persist unchanged for up to a month following termination of drug exposure (Miller et al., 2001; 2003), with one study reporting observed effects at 75 days post termination of treatment (Villegier et al., 2003). The persistence of the increased responsivity to nicotine's effects on disinhibition provides strong support that a loss of inhibitory control may be a key mechanism underlying smoking relapse (Robinson and Berridge, 2000; DeFranza and Wellman, 2005). The findings suggest that an abstaining smoker that is exposed to even low levels of nicotine is at a substantial risk of relapsing due to the profound effect of nicotine on the loss of inhibitory control over future drug seeking and taking behaviour. In support for this theory one of the strongest predictors of smoking relapse is the initial lapse of smoking one cigarette following quitting (Brandon et al., 1990; Nides et al., 1995; Shiffman et al., 1996), findings which can now possibly be attributed to the induced profound loss of control over behaviour

following the acute exposure to nicotine.

The neurobiological processes believed to be involved in nicotine sensation may potentially be mediating the enhanced drug induced disinhibition. Chronic stimulation of the mesocorticolimbic DA system is believed to lead to neural adaptations pre and postsynaptically rendering the system hypersensitive to the effects of nicotine (Robinson and Berridge, 1993). These alterations led to the augmented release of DA in the NAc (Cadoni and Di Chiara, 2000; Olausson et al., 2001a; Rahman, Zhang and Corrigall, 2003) and PFC (Vezina et al., 1992; Nisell et al., 1996) following subsequent exposure nicotine. As discussed previously, a crucial role for DA receptor activation in stimulant drug induced disinhibition has been provided by recent research (Van Gaalen et al., 2006; Pattij et al., 2007). Research conducted by Olausson and colleagues has furthermore demonstrated that sensitisation to nicotine may depend on a complex interaction between dopaminergic and serotonergic systems. Adopting the elevated plus maze, authors demonstrated that rats repeatedly treated with nicotine spent an increasingly greater amount of time on the open arms and made more entries to the open arms of the maze (Olausson et al., 1999; Olausson et al., 2001b). Although traditionally an ethological model of anxiety, the researchers tentatively argued that the disinhibited behaviour in the maze could also be a reflection of an increased sensitisation to nicotine's effects on impulsive control (Olausson et al., 1999; Olausson et al., 2001b; Olausson, Engel and Soderpalm, 2002). Interestingly, subchronic treatment with both the SSRI citalopram, and the 5-HT_{1A} agonist 8-OH-DPAT blocked the sensitised expression of disinhibited behaviour in the maze. These findings suggest that enhanced disinhibition following chronic nicotine treatment may depend on nicotine induced reduction in 5-HT neurotransmission (Olausson et al., 1999; Olausson et al., 2001b). Although it is important that these findings are replicated in a validated model of impulsivity such as go/no-go task, the findings importantly indicate the potential of serotonergic enhancing drugs as future pharmacological agents that may aid smoking cessation. Indeed, it has previously been shown in rodents that comparable 5-HT enhancing drugs are capable of attenuating the self administration of psychostimulants including nicotine (Optiz and Weisner, 1988), cocaine (e.g. Carroll et al., 1990; Glatz et al., 2002) and amphetamine (Lyness, 1983), possibly by means of strengthening inhibitory control. Furthermore, in human research the SSRI paroxetine when administered in combination with transdermal nicotine patches increased abstinence rates significantly (Killen et al., 2000). These findings taken together with the presented evidence of the crucial role disinhibition may have in smoking relapse, warrants greatly further investigation of the effectiveness of 5-HT enhancing agents as treatment for nicotine dependence.

5.4.5. Conclusion

To conclude, the series of studies within this chapter has yielded extensive evidence to suggest

that the association between smoking and disinhibition is partly attributed to nicotine induced effects on inhibitory control. Chronic nicotine administration led to a specific reduction in inhibitory control in the go/no-go paradigm; an effect that was not sustained over the seven days of treatment, suggesting that tolerance to the effects of nicotine had developed. Termination of nicotine treatment caused a spontaneous nicotine abstinence syndrome, during which inhibitory control over behaviour appeared to be enhanced. This improvement in impulsive control was however short lived with a contrasting deficit in inhibitory control observed during longer term withdrawal. Three weeks following termination of treatment baseline levels of inhibitory control returned to pre-treatment levels of impulsivity, confirming that chronic nicotine induced effects on inhibitory control is a reversible effect. The neural adaptations associated with chronic exposure however rendered animals sensitised to the effects of acute nicotine on inhibitory control, highlighting the potential relevance of disinhibition in smoking relapse. Taken together, these findings suggest that a loss of inhibitory control induced by nicotine may be critically involved in both the initial and end stages of nicotine addiction. More specifically, exposure to nicotine in the early stages of abuse may lead to a loss of control over future drug seeking and taking behaviour increasing the likelihood of the transition to addiction and maintenance of the disorder. With increasing drug exposure however, tolerance to nicotine's influence on inhibitory control is likely to develop. The neural adaptations that then develop as a consequence of chronic exposure may become unmasked during long term abstinence giving rise to dysfunctional inhibitory systems leading to a loss of control over behaviour and subsequent relapse at the end stages of addiction. Whilst discussion has been made of the potential neurobiological events that may have mediated the behavioural effects observed, it is important that future research begins to determine conclusively the processes involved. Such research will hopefully in turn enable progression of the development of future pharmacological treatments that may aid smoking cessation.

CHAPTER 6

Validation of the delayed reward paradigm

6.1. INTRODUCTION

6.1.1. General Introduction

Impulsivity has been equated with the preference of immediate over delayed gratification (Rachlin and Green, 1972; Ainslie, 1974). This particular subcomponent of impulsivity contrasts to that of behavioural disinhibition in that impulsive choice reflects more a cognitive, decision-making process rather than motor inhibition (Brunner and Hen, 1997; Evenden, 1999). One of the most common and effective behavioural assessments of impulsive choice is delay discounting, which is based upon the function by which the subjective value of a reward is depreciated by a delay to its delivery (Green et al., 1994; Bickel and Marsh, 2001). Research has revealed that both humans and non-humans discount the value of rewards as the delay to its receipt increases, with the loss of value of delayed reward being faster in more impulsive subjects (Mazur, 1987; Rachlin et al., 1991; Green et al., 1994; Richards et al., 1997). Both exponential, and to a greater extent hyperbolic, functions have accounted for the trend of discounting of delayed reward across humans and animals (e.g. Kirby, 1997; Richards et al., 1997; 1999a; Mazur, 2001; Kirby and Santiesteban, 2003; Frederick et al., 2003). The similarity in discounting processes across species is of importance as it potentially allows researchers to extrapolate findings from non-human models to humans.

The various paradigms utilised to measure delay discounting in both humans and animals have been detailed previously (section 1.3.2.2.). Rat models of impulsive choice can be divided into “systematic” and “adjusting” delayed reward tasks. The present thesis adopted the former of these procedures, which requires food-restricted animals to choose between one of two levers; one delivering a single food pellet immediately, the other delivering several pellets after a programmed delay. Choices are made during discrete trials and the delay to the delivery of the larger reinforcer is increased (or decreased) systematically as the session progresses, normally from 0 to 60 seconds (e.g. Evenden and Ryan, 1996; Cardinal et al., 2000). Percent choice of delayed reward is taken as an index of impulsive choice, with more impulsive animals choosing the delayed reward to a lesser extent. Research has reliably demonstrated that, at baseline, animals’ choice behaviour in the task is highly sensitive to delay; and task manipulations that reduce or omit standard delays to the delivery of the larger reward result in an increased preference of the delayed, larger reward (Evenden and Ryan 1996; Cardinal et al., 2000; Isles et

al., 2003).

6.1.2. Stability of Impulsive Choice

Despite the extensive use of the delayed reward task in preclinical research (e.g. Evenden and Ryan 1996; 1999; Cardinal et al., 2000; Paine et al., 2003; Winstanley et al., 2005), surprisingly limited investigation has been made regarding the stability of choice behaviour in the task. In order to assess the long-term effects of chronic nicotine administration and subsequent nicotine withdrawal on impulsive choice, it is essential that the stability of baseline impulsivity in the task is established in the absence of drug treatment and withdrawal. Previous research has provided evidence that animals' choice behaviour remained delay-sensitive across many months of study; however no indication was given of whether levels of impulsive choice changed over time (Evenden and Ryan, 1996). More recently, research has indicated extensive individual variation in levels of impulsive choice in rodents, as measured by systematic delayed reward paradigms (Cardinal et al., 2000; Winstanley et al., 2003a; Deltu-Hagedorn, 2006). Only Winstanley et al., (2003), however, demonstrated evidence of stability of choice behaviour prior to pharmacological manipulations. Choice behaviour of individual animals was reported to be stable across a 10 day period, irrespective of the level of impulsivity.

6.1.3. The Effects of Alterations in Primary Motivation on Impulsive Choice

As previously discussed in Chapter 3, as many drugs of abuse such as nicotine, have appetitive effects (e.g. Grunberg, 1986; Miyata et al., 1999; Zhang et al., 2001), it is essential to demonstrate that delay discounting is not altered by changes in primary motivation. As food rewards are utilised in the delayed reward task, nicotine-induced changes on performance could arguably be due to alterations in primary motivation rather than sensitivity to delayed gratification. To date, research has yielded inconsistent effects of varying levels of motivation on delay discounting in animals. Both an increase in choice of delayed reward (Bradshaw and Szabadi, 1992; Wogar 1992; Ho et al., 1997) and lack of effect have been reported following the increase in primary motivation (Logue and Pena-Correal 1985; Logue et al., 1988; Richards et al., 1997; Cardinal et al., 2000; Wade et al., 2000). In contrast to this, no effects on preference for delayed reward have been reported following a reduction in deprivation state (Logue and Pena-Correal, 1985; Logue et al., 1988; Richards et al., 1997; Cardinal et al., 2000; Wade et al., 2000).

6.1.4. Objectives of Experiment 5

The studies presented in this chapter were designed to assess the suitability of the delayed reward task for later experiments investigating acute and long-term nicotine administration and nicotine withdrawal. To achieve this, a series of experiments were conducted in a control group of animals to assess:

- i) The stability of performance in the delayed reward task (Experiment 5A).
- ii) The effects of acute alterations in primary motivation on task performance (Experiment 5B).
- iii) The effects of prolonged alterations in primary motivation on task performance (Experiment 5C).
- iv) The sensitivity of choice behaviour to alterations in the delay to the delivery of reward (Experiment 5D).

These objectives were achieved through a series of preliminary control studies. In the first of these studies, the stability of task performance of a control group of animals was assessed across a three week period. Individual differences in levels of impulsive choice were also determined through the application of the exponential delay discounting function to choice data. The acute effects of alterations in primary motivation on task performance were then assessed by altering the level of motivation for food reward through either prefeeding subjects immediately prior to the session or acutely increasing levels deprivation - manipulations known to effectively vary the value/motivation for food reward (Heyman and Monaghan, 1987). Based on the limitations of Chapter 3, examination was also made of the effects of prolonged changes in deprivation level on performance in order to more effectively compare this study with long-term drug studies. Finally, to examine the sensitivity of choice behaviour to delay exploration was made of the effects of a reduction in duration of normal task delays on task performance during a single test session.

6.2. EXPERIMENT 5 (A-D): VALIDATION OF THE DELAYED REWARD TASK: PRELIMINARY CONTROL STUDIES

6.2.1. METHOD

6.2.1.1. Subjects

Subjects were 10 adult male Lister-hooded rats (Charles River, UK) weighing 360-390g at the start of training. On arrival to the laboratory animals were housed in pairs (cage size; 46 X 26.5 X 26cm) and maintained under a 12 hour light/dark cycle (lights on at 0700h; lights off at 1900h) at a controlled environmental temperature of $21.5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity of $55\% \pm 5$. Animals were maintained at 85% of their free feeding adult body weights throughout the testing period. Water was available ad libitum in home cages and feeding occurred at the end of each experimental day. The same subjects were utilised for Experiments 5(A-D). All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986.

6.2.1.2. Apparatus

Four identical operant chambers were used (dimensions 30.5 X 24.1 X 21cm and 30.5 X 24.1 X 29.2cm; Med Associates Inc., USA). Refer to General Methodology of Chapter 2, section 2.5.1,

for a detailed description of apparatus.

6.2.1.3. Behavioural Testing: Delayed Reward Task

6.2.1.3.1. Pre-training

Animals were initially magazine trained by placing sucrose pellets in the illuminated magazine and allowing free access to them for two consecutive sessions. CRF lever training then commenced, during which, animals were trained to lever press the right and left lever on alternate training sessions. Responding on a lever resulted in the illumination of the magazine light and the delivery of a single sucrose pellet. CRF training, on each lever, continued until 100 pellets were earned within a 20-minute period on two consecutive sessions. This normally required no more than five training sessions.

Animals were then trained on a simplified version of the full delayed reward task. Each trial was 40 seconds in duration and commenced with the illumination of the house and magazine light and retraction of both levers. Animals were required to enter the magazine within 10 seconds in order for a single lever to be presented and the magazine light to be turned off. If the animal failed to nose poke the magazine then the trial was aborted and the chamber returned to darkness for the remainder of the current trial. When presented with a lever, a response was required within 10 seconds or the chamber returned to darkness and the lever retracted until the following trial was initiated. If the animal did respond on the lever, a single pellet was delivered and the magazine light was turned on until either the pellet was collected or six seconds had passed. Trials were presented in pairs so that the left lever was presented once and the right lever presented once. The order of presentation during each pair of trials was randomised. This stage of training continued until 60 trials had been successfully completed within one hour for two consecutive sessions. Animals were then transferred to the full task where delays and small verses larger rewards were introduced.

6.2.1.3.2. Delayed Reward Task

The delayed reinforcement task was based on Evenden and Ryan (1996) and Cardinal et al. (2000) behavioural procedures and is illustrated in Fig 6.1. The task consisted of a total of 60 trials, each of 100 seconds duration. The initiation of each trial was signalled by the illumination of the house light and magazine light. Animals were required to nose poke the magazine within 10 seconds, which resulted in the magazine light being extinguished and both levers being presented. This ensured that the animal was centrally located between both levers prior to making a response choice. The latency to enter the magazine was recorded as the trial initiation latency. If the animal failed to make a nose poke response during this time period the trial was recorded as a “trial” omission and the chamber returned to darkness. The chamber remained in this ITI period for the remainder of the trial duration.

On successful presentation of the levers, animals were required to respond on either lever within a 10 second period. If no lever response occurred, the levers were retracted, the houselight extinguished and the chamber returned to the ITI for the remaining duration of the trial. This behaviour was then recorded as a “choice” omission. One lever was designated the ‘immediate’ lever the other the ‘delayed’ lever for each animal. Assignment of ‘immediate’ and ‘delayed’ levers was counterbalanced left or right across animals. When a response on the lever was made both levers were withdrawn and the houselight turned off. A response on the ‘immediate’ lever resulted in the immediate delivery of a single food pellet. A response on the ‘delayed’ lever resulted in the delivery of five food pellets, delivered one second apart, following a programmed delay (of either 0, 10, 20, 40, 60 seconds). The latency to choose a lever was recorded as the response latency and was measured from the point of initiating the trial, by nose poking the magazine, to pressing the lever. During the delivery of sucrose pellets the magazine light was illuminated until either the animal entered the magazine to collect the reward or six seconds had passed. The chamber then entered the ITI period for the remainder of the 100 seconds until the initiation of the following trial. Initiation and response latencies were used to calculate the length of the ITI period to ensure that, regardless of behaviour in each trial, the duration of all 60 trials remained constant. If collection of the pellets occurred prior to the initiation of the next trial then magazine latency was calculated from the time of delivery of the first pellet until point of entrance into the magazine to collect the reward. Failure to collect pellets prior to the next trial was recorded as a “magazine” omission.

Each session comprised of a total of 60 trials consisting of five blocks of 12 trials. Delays varied across blocks so for each block delays were 0, 10, 20 40 and 60 seconds. The direction of delays was counterbalanced across animals, so that for half the animals delays ascended in a step-wise manner across the session, and for the remaining, descended. Each block of trials began with two forced trials followed by 10 free-choice trials. During forced trials only one lever was presented, therefore no choice was available. Each lever was presented once in a randomised order during one of the forced trials. Implementing forced trials ensured that the animal had been exposed to the delay and that they also experienced both the large and small rewards prior to making a choice in the following free choice trials. During free choice trials both levers were presented. Each session length was 100 minutes and animals received one session per day.

Immediate and delayed lever choices were recorded during free choice trials - allowing percent choice of delayed lever to be calculated across delay (no. of delayed lever responses/total number of responses). Impulsive choice can be measured as a decrease in choice of the delayed lever. Omissions were excluded from choice calculations. Table 6.1 summarises all dependent measures recorded in the procedure. Training continued until animals displayed stable, delay-

dependent behaviour across three consecutive sessions. This required approximately 10 weeks of training.

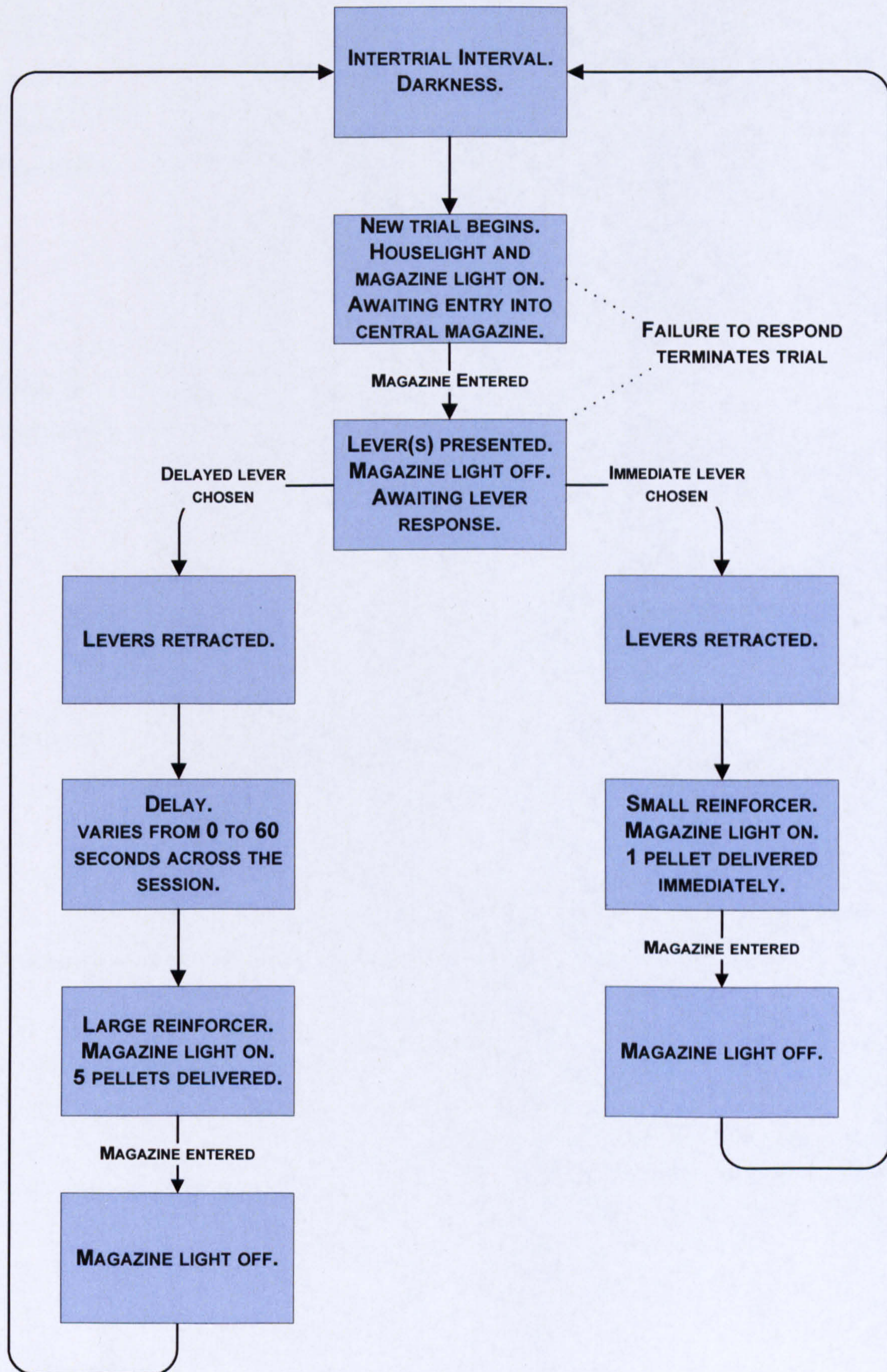


Fig. 6.1: Schematic diagram of the Delayed Reward Task (adapted from Cardinal et al., 2000)

Table 6.1: Behavioural measures recorded in the delayed reward task

Behavioural Measure	Dependent Variables
Choice Behaviour	<ul style="list-style-type: none"> • Overall percent choice of delayed lever (No. of delayed reward lever responses/ (50-no. of omissions)*100). • Percent choice of delayed lever by delay (0, 10, 20, 40 & 60) (No. of delayed reward lever responses/ (10-no. of omissions)*100).
Speed of Responding	<ul style="list-style-type: none"> • Trial initiation latency (seconds) • 'Delayed' reward lever response latency (seconds) • 'Immediate' lever response latency (seconds) • Magazine latency following delayed reward choice (seconds) • Magazine latency following immediate reward choice (seconds)
Omissions	<ul style="list-style-type: none"> • No. of trial omissions • No. of choice omissions • No. of magazine omission following delayed choice • No. of magazine omission following immediate choice

6.2.1.4. Design and Procedure

All experiments followed standard operant testing procedures, previously outlined in detail in the general methodology of Chapter 2, section 2.5.2. Experimentation took place over a 16 week period. Experiments 5 (A-D) employed a within-subjects design. Prior to all subsequent experimental manipulations it was ensured that animals had returned to BL performance prior to further testing. BL was defined in the task as impulsive choice deviating no greater than 10% from the level of overall choice of delayed reward demonstrated by the subject during the assessment of stability in Experiment 5A. All testing took place during the light phase of their LD cycle between 0800h and 1730h. Time of operant testing remained constant for each subject across experiments.

6.2.1.4.1. EXPERIMENT 5A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE DELAYED REWARD TASK

Once trained, the stability of choice behaviour, speed of responding and frequency of omissions in the task were examined across a three week period. The duration of assessment was chosen to reflect the time period of future chronic drug studies, where performance would be examined during seven days of baseline, chronic drug treatment and drug withdrawal.

6.2.1.4.2. EXPERIMENT 5B: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

Following Experiment 5A, the acute effects of varying levels of deprivation on task performance was assessed. The effect of a decrease in motivation for food reward on behavioural parameters in the task was assessed twice using different procedures. The experimental conditions differed in terms of whether subjects were fed normal rat chow or sucrose reward pellets prior to the session. Under the first of these conditions motivation for reward was suppressed through allowing free access to a pre-weighed amount of normal rat chow for one hour immediately prior to operant testing in the subject's home cage. Under the second condition, motivation for sucrose reward was reduced through allowing animals' free access to a pre-weighed amount of sucrose pellets for a 30 minute period prior to testing. The latter of these conditions enabled the reduction of specific satiety for the sucrose reward that would be earned during the test session. A shorter duration of free access to sucrose pellets was selected based on preliminary observations that indicated a much greater and rapid consumption of sucrose pellets in comparison to normal rat chow. For both experimental manipulations, animals were individually housed. In order to quantify the level of presatiation, each animal's BW was measured prior to and after feeding. The amount of food consumed during the prefeeding periods was also recorded.

The third manipulation explored the effects of increasing motivation for food reward on performance in the task. This was achieved through the 50% reduction of allocated food allowance (normally 18.6g) the day prior to testing. The following test day animals were weighed immediately prior to operant testing to confirm loss of BW. The sequence of manipulations for each subject was determined by a Latin square. Between each manipulation, an inter-test interval of at least six days elapsed, during which, animals were maintained under their normal BL testing and feeding conditions.

6.2.1.4.3. EXPERIMENT 5C: EXAMINATION OF THE EFFECTS OF CHRONIC ALTERATION IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

Following Experiment 5B, assessment was made of chronic alterations in primary motivation on task performance. This was achieved through altering daily food ration (normally 18.6g, inclusive of food reward consumed in the task). To assess a longer term decrease in motivation, the daily food ration was increased by 20% over a seven day period. To assess a longer term increase in motivation, the daily food ration was decreased by 20% over a seven day period. This duration of assessment was chosen to reflect the time period over which chronic nicotine and nicotine withdrawal would be assessed in the task. Between each manipulation, an inter-test interval of at least six days elapsed, during which, animals were maintained under their normal BL testing and feeding conditions.

6.2.1.4.4. EXPERIMENT 5D: EXAMINATION OF THE REDUCTION OF DELAYS TO THE DELIVERY OF REWARD ON PERFORMANCE IN THE DELAYED REWARD TASK

To provide further support that choice behaviour in the task was delay sensitive, within a single test session, normal delays (0, 10, 20, 40 and 60 seconds) were replaced with reduced delays of 0, 1, 2, 5 and 15 seconds. All other aspects of the task remained identical, with animals completing 12 trials during each delay condition, the first two of which were forced choice trials. This enabled the direct comparison of choice behaviour during delay conditions with normal and reduced delays.

6.2.1.5. Statistical Analysis

6.2.1.5.1. Training

To determine that animals' choice behaviour in the task was both sensitive to delay and stable prior to the initiation of testing, data from three consecutive test sessions was analysed using a two-way repeated measures ANOVA, with both day and delay (0, 10, 20, 40 and 60s) as the within-subject factors. A significant main effect of delay would indicate sensitivity of choice behaviour across delays, while a non-significant main effect of day would demonstrate evidence of stability of this choice.

6.2.1.5.2. EXPERIMENT 5A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE DELAYED REWARD TASK

To assess the stability of behaviour in the task, overall choice of delayed reward, speed of responding and omissions were analysed using a one-way repeated measures ANOVA, with test day as the within-subject factor. Analysis of the choice of reward by delay, was conducted using

a two-way repeated measures ANOVA, with both test day and delay (0, 10, 20, 40 and 60s) as the within-subject variables.

Extensive variability in the sensitivity of delayed reward was identified within the group of animals tested. In order to further explore the individual variation in choice behaviour within the task, based on Winstanley et al. (2005; 2003a), the level of impulsive choice for each animal was determined through the application of the exponential curve to the averaged data obtained during assessment of stability, in the form:

$$y = e^{-(kd)}$$

Where:

y = the frequency of choice of the delayed larger reward.

d = delay in delivery of the large reward.

k = rate of exponential decrease of choice of the larger reward with increasing delay.

The larger the **k** value, the steeper the exponential curve, indicating that animals with increasing delay chose the smaller immediate reward to a greater extent. Highly impulsive animals would therefore display greater **k** values in comparison to less impulsive. R^2 was utilised as an index of the exponential equation's goodness of fit of the animal's choice data. The application of exponential curves was conducted using Microsoft Excel Curve fitting programme.

6.2.1.5.3. EXPERIMENT 5B: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

For each motivational manipulation, all behavioural measures in the task were compared to the prior averaged five day baseline performance. With the exception of choice by delay, analysis of all parameters within the task was conducted using a repeated measures t-test. Analysis of the choice of reward by delay, in contrast, was analysed by a two-way repeated measures ANOVA, with delay and motivational level as the within-subject factors. Comparisons of differences in food intake and weight gained across the prefeeding manipulations were also conducted by a repeated measures t-test. Weight was assessed as both an absolute weight gain or loss (g) and as percentage change from the weight recorded immediately prior to the free feeding period.

6.2.1.5.4. EXPERIMENT 5C: EXAMINATION OF THE EFFECTS OF CHRONIC ALTERATION IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

Performance on the task during prolonged alterations in motivation for food reward were compared to BL behaviour averaged across the five days immediately prior to initiation of the manipulation. Overall choice of delayed reward, speed of responding and omissions were

analysed by a one-way repeated measures ANOVA, with day as the within-subject factor with eight levels (BL, plus seven test days). Choice by delay was analysed by a two-way repeated measures ANOVA, with day and delay as the within-subject factors. Changes in BW during chronic alterations in motivation were expressed as both absolute weight gain or loss (g) and percentage change in BW from the day prior to testing. These data were compared to changes in BW during the BL week, prior to the initiation of testing by repeated measures t-tests.

6.2.1.5.5. EXPERIMENT 5D: EXAMINATION OF THE REDUCTION OF DELAYS TO THE DELIVERY OF REWARD ON PERFORMANCE IN THE DELAYED REWARD TASK

Task performance during this manipulation was compared to BL. BL behaviour was defined as average task performance across the five days prior to testing. Overall choice of delayed reward, speed of responding and omissions were analysed by repeated measures t-tests. Due to differing task delays, statistical comparison of choice by delay was no longer appropriate between BL and the task manipulation. Delay dependency was therefore analysed independently for BL and the delay manipulation. This was achieved by a one-way repeated measures ANOVA, with delay as the within-subject factor.

For Experiments 5 (A-D), all significant main effects were followed, where appropriate, by Bonferroni post hoc comparisons. Significant interactions observed following the analysis of choice by delay were examined further by simple effects analysis. Repeated measures one-way ANOVAs (Experiments 5A and 5C) or t-tests (Experiments 5B) were used to examine differences in choice behaviour at each delay (Bonferroni correction of $p < 0.01$). The interactions were also examined by a one-way ANOVA assessing the main effect of delay under each level of the variable being tested.

For all statistical procedures, data prior to analysis were assessed for normality and transformed where necessary (see also section 2.7). Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom were adjusted with the Greenhouse-Geisser correction. If data could not be successfully transformed, then the non-parametric equivalent - Friedman and Wilcoxon Signed Ranks tests - were employed. In all cases of statistical analysis, α was set at $p < 0.05$.

6.3. RESULTS

Across Experiments 5 (A-D) omissions of both lever selection, and collection of reward following an immediate choice, were not analysed due to both behaviours rarely occurring.

6.3.1. Training

Animals' choice behaviour in the task became sensitive to delay following an average of $42.5 \pm$

2.06 training sessions. Choice behaviour was both delay sensitive ($F(4, 36) = 33.106, p < 0.001$) and stable ($F(2,18) = 2.372, N.S.$) prior to testing. Post hoc analysis revealed that the greatest sensitivity to delay was observed at 20, 40 and 60 second delay conditions, during which preference for the delayed reward was significantly reduced in comparison to 0 delay choice trials (all $p < 0.05$). Analysis demonstrated no significant delay x day interaction ($F(8,72) = 1.173, N.S.$)

6.3.2. EXPERIMENT 5A: EXAMINATION OF THE STABILITY OF BEHAVIOUR IN THE DELAYED REWARD TASK

6.3.2.1. Choice Behaviour

Figure 6.2 illustrates the overall percentage choice of the delayed larger reward across the 21 day period. Analysis demonstrated that overall choice behaviour was stable across this time period with no significant main effect of test day ($F(20, 180) = 1.374, N.S.$). Analysis of choice behaviour by delay supported further the stability of performance with no significant main effect of test day ($F(5.528, 49.751) = 1.349, N.S.$). A significant main effect of delay was however found, demonstrating that choice behaviour was highly sensitive to delay ($F(1.977, 17.795) = 47.164, p < 0.001$). As clearly shown in Fig. 6.3, preference of the delayed reward decreased with increasing delays. The delay-dependent effect was supported by post hoc analysis that revealed choice behaviour differed significantly between all delay conditions (all $p < 0.05$). An exception to this effect was the reduction in choice during the 10 second delay trials which failed to reach significance in comparison to the 0 second delay condition during the task ($p > 0.05$). Analysis further revealed no significant test day x delay interaction during the 21 days ($F(8.092, 72.824) = 1.507, N.S.$).

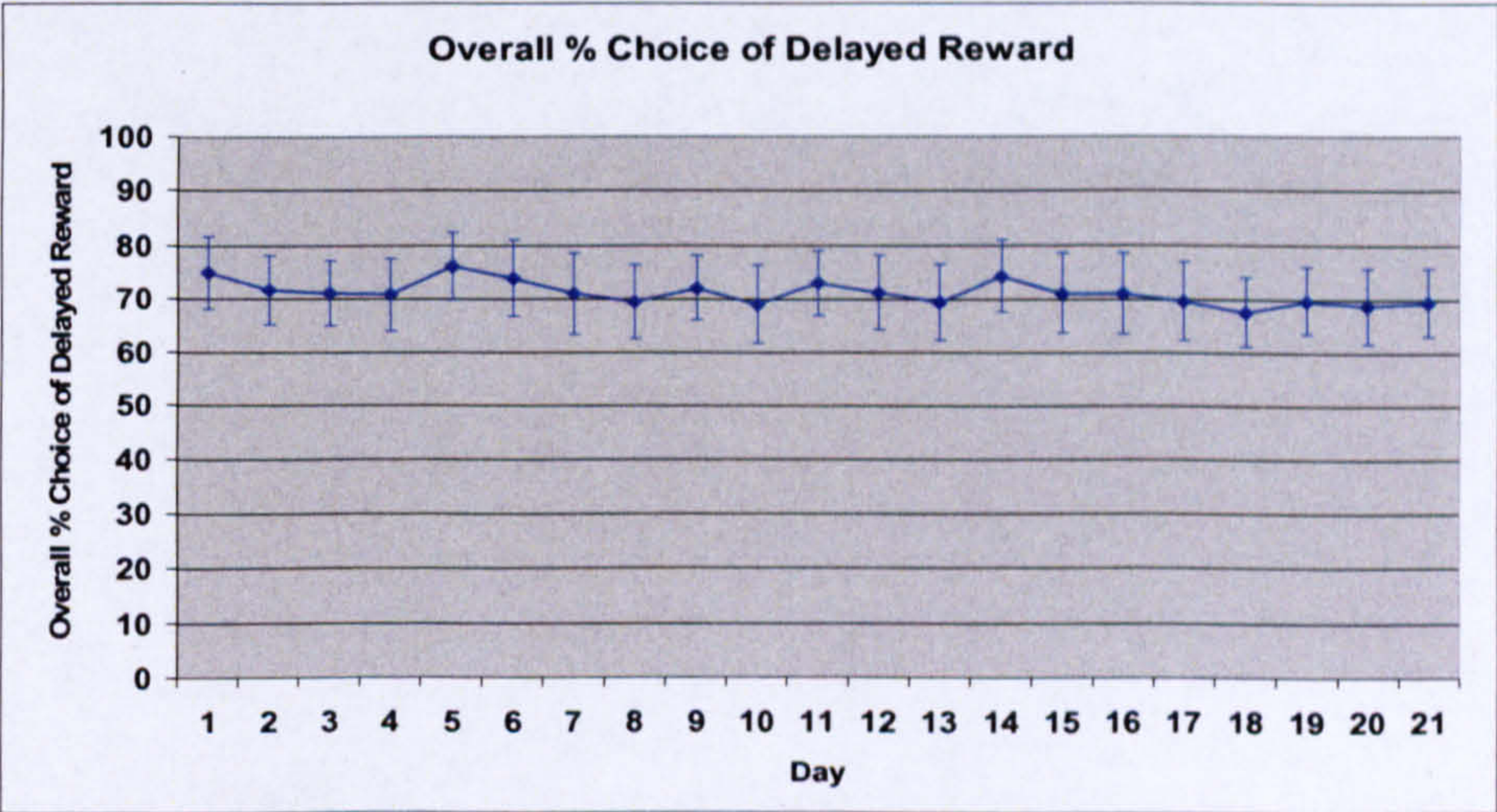


Fig. 6.2: Choice Behaviour: stability of overall percentage choice of delayed reward across the 21 day period. Each point represents the mean percentage ± SEM.

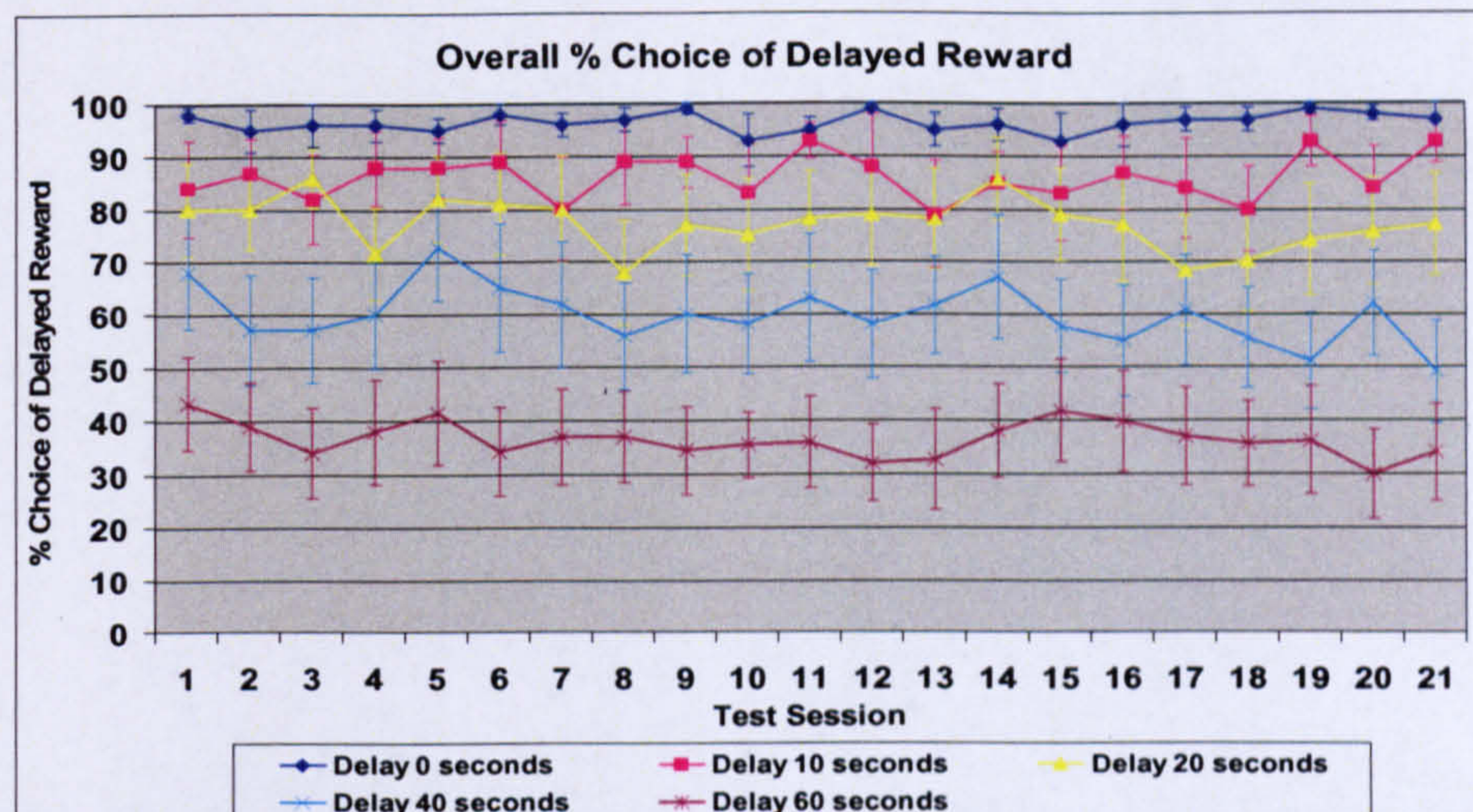


Fig. 6.3: Choice Behaviour: stability of percentage choice of delayed reward during each delay across the 21 day period. Each point represents the mean percentage \pm SEM.

6.3.2.2. Speed of Responding

Figure 6.4 (a-e) illustrates the stability of speed of responding measures in the delayed reward task across the 21 day period. Analysis revealed no significant main effect of test day on the latency to initiate trials ($F(3.617, 32.549) = 1.047$, N.S.) and the speed with which the delayed and immediate reward were chosen ($F(3.374, 30.366) = 2.580$, N.S.; $F(4.028, 36.248) = 2.570$, N.S., respectively).

The speed with which reward was collected following an immediate choice was also stable across testing ($F(1.105, 9.946) = 1.579$, N.S.). As shown in Fig. 6.4 (e), the latency to collect reward following a delayed reward choice was both greater and more variable across animals than immediate reward magazine latency. However, there was no significant main effect of test day on this measure ($F(4.613, 41.513) = 0.479$, N.S.).

6.3.2.3. Omissions

Failure to both initiate trials and collect food reward following a delayed reward choice remained stable across the 21 day period ($X^2 = 21.559$, $df = 20$, N.S.; $X^2 = 11.838$, $df = 20$, N.S., respectively) (see Fig. 6.5).

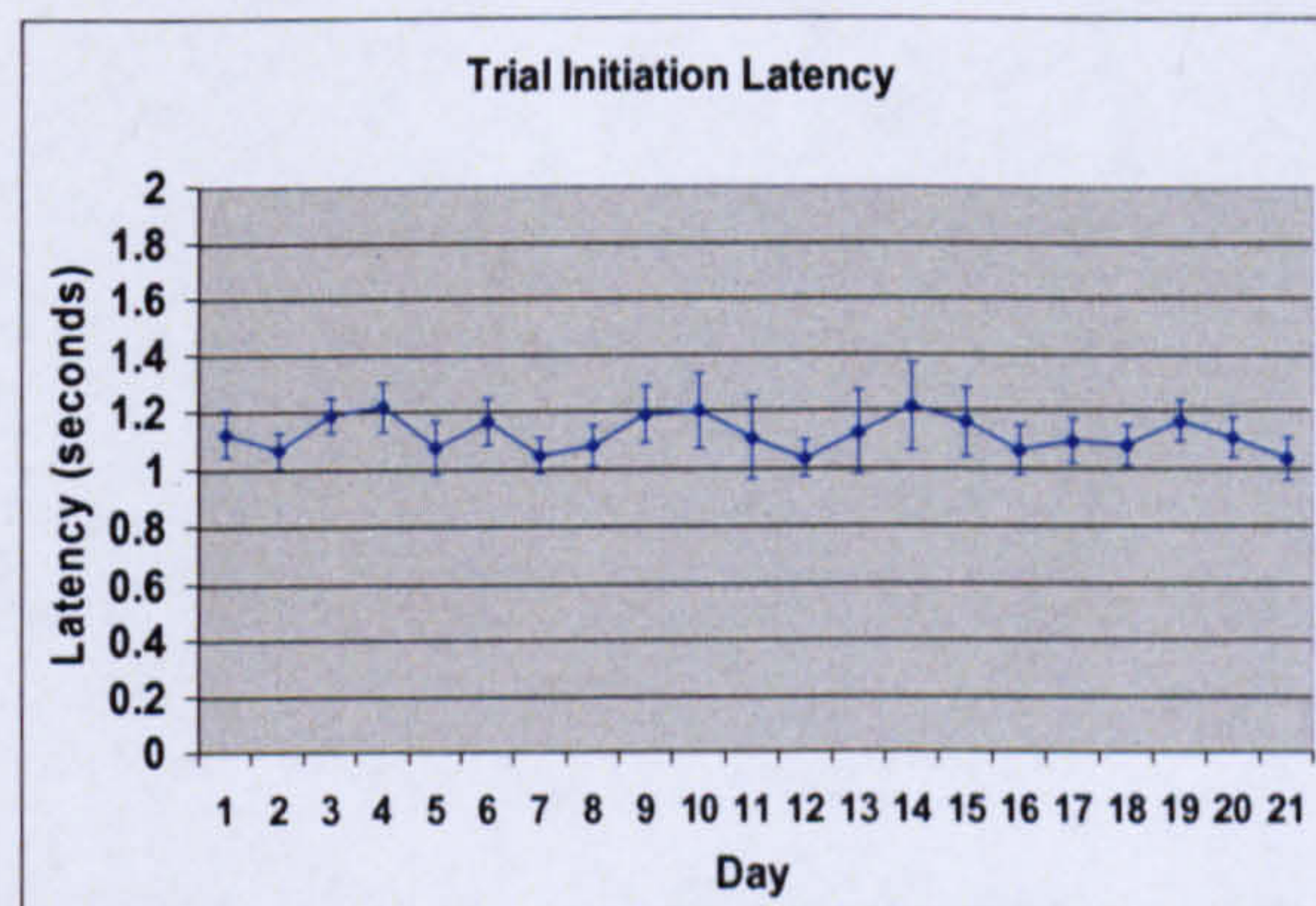
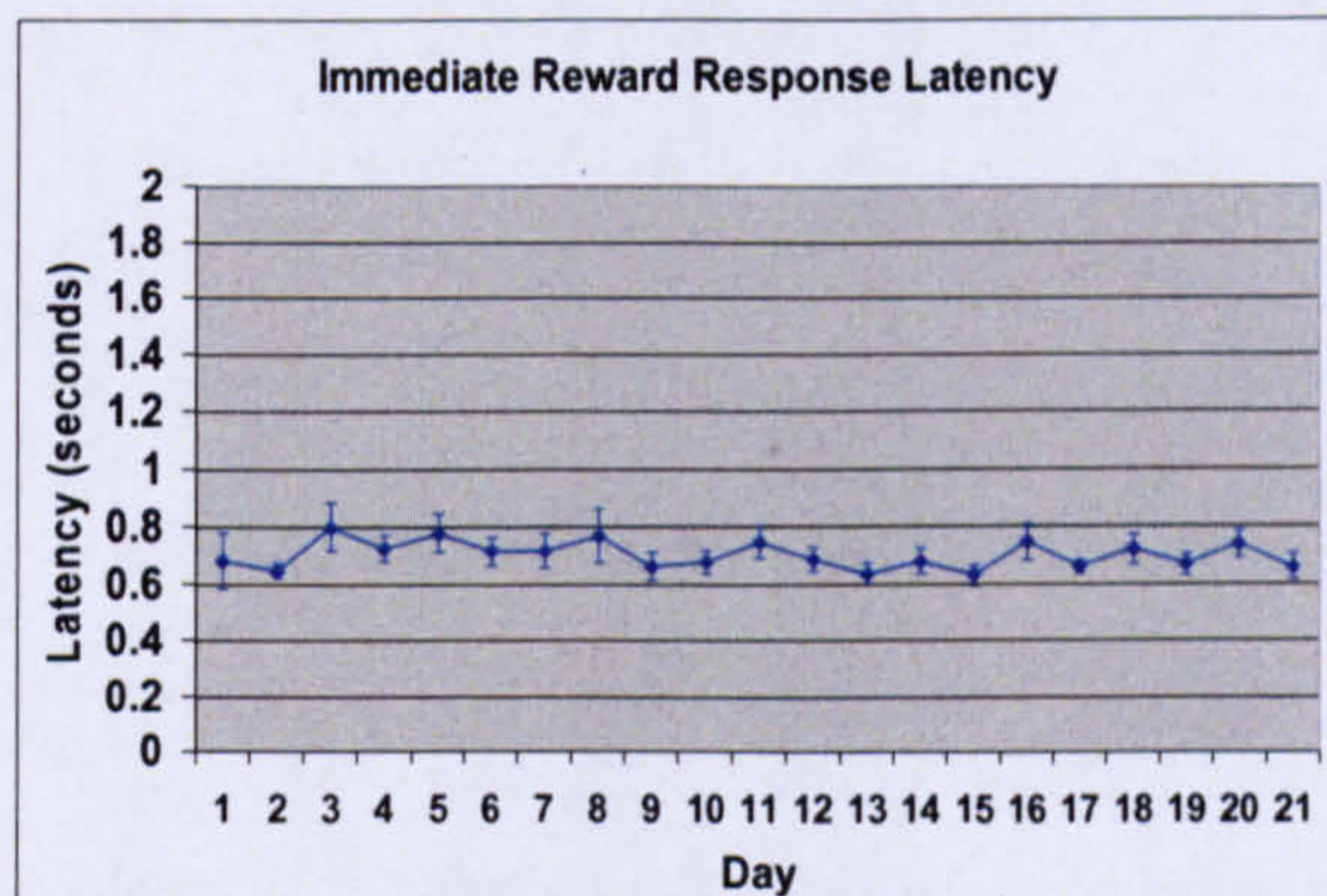
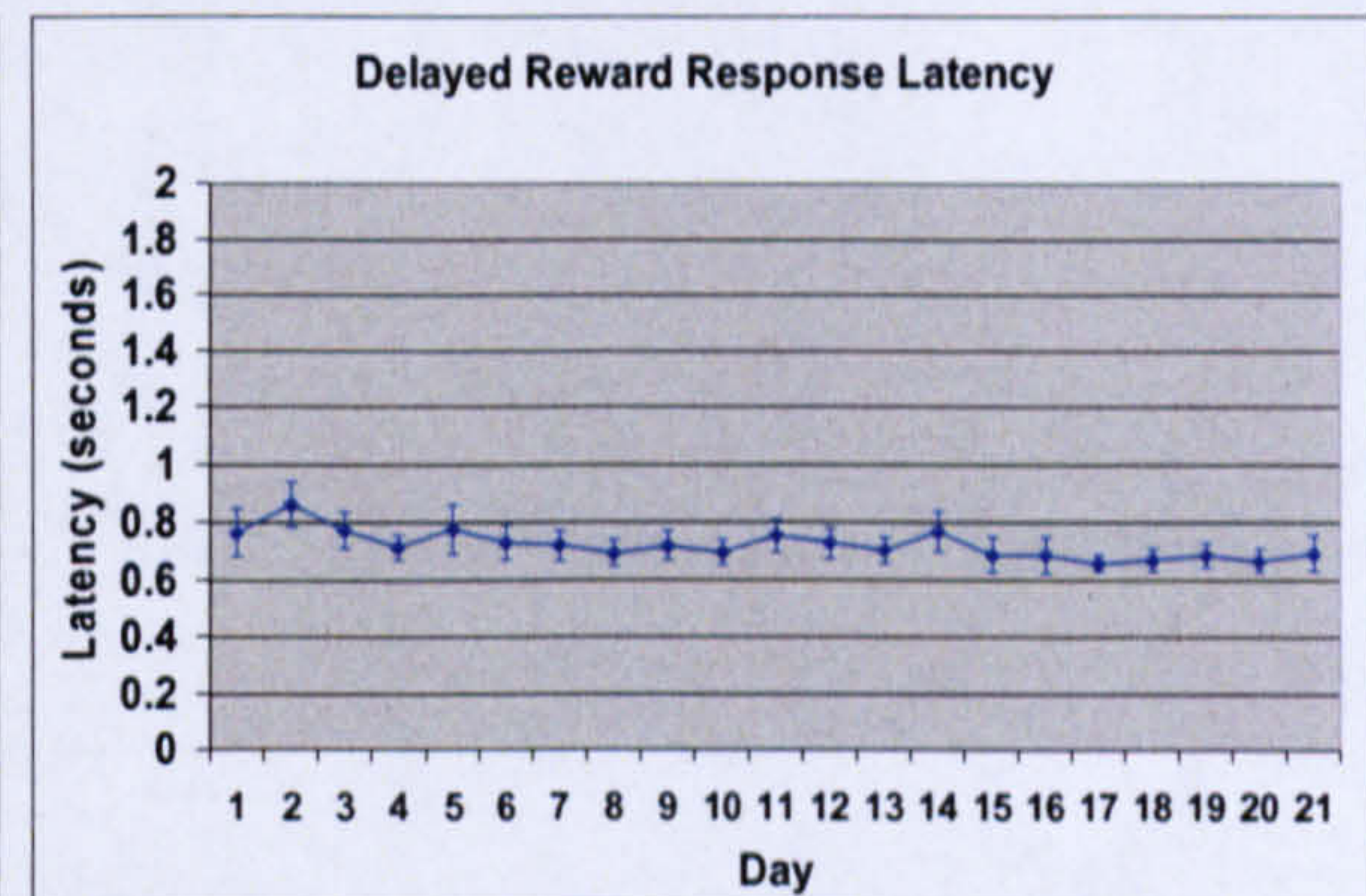
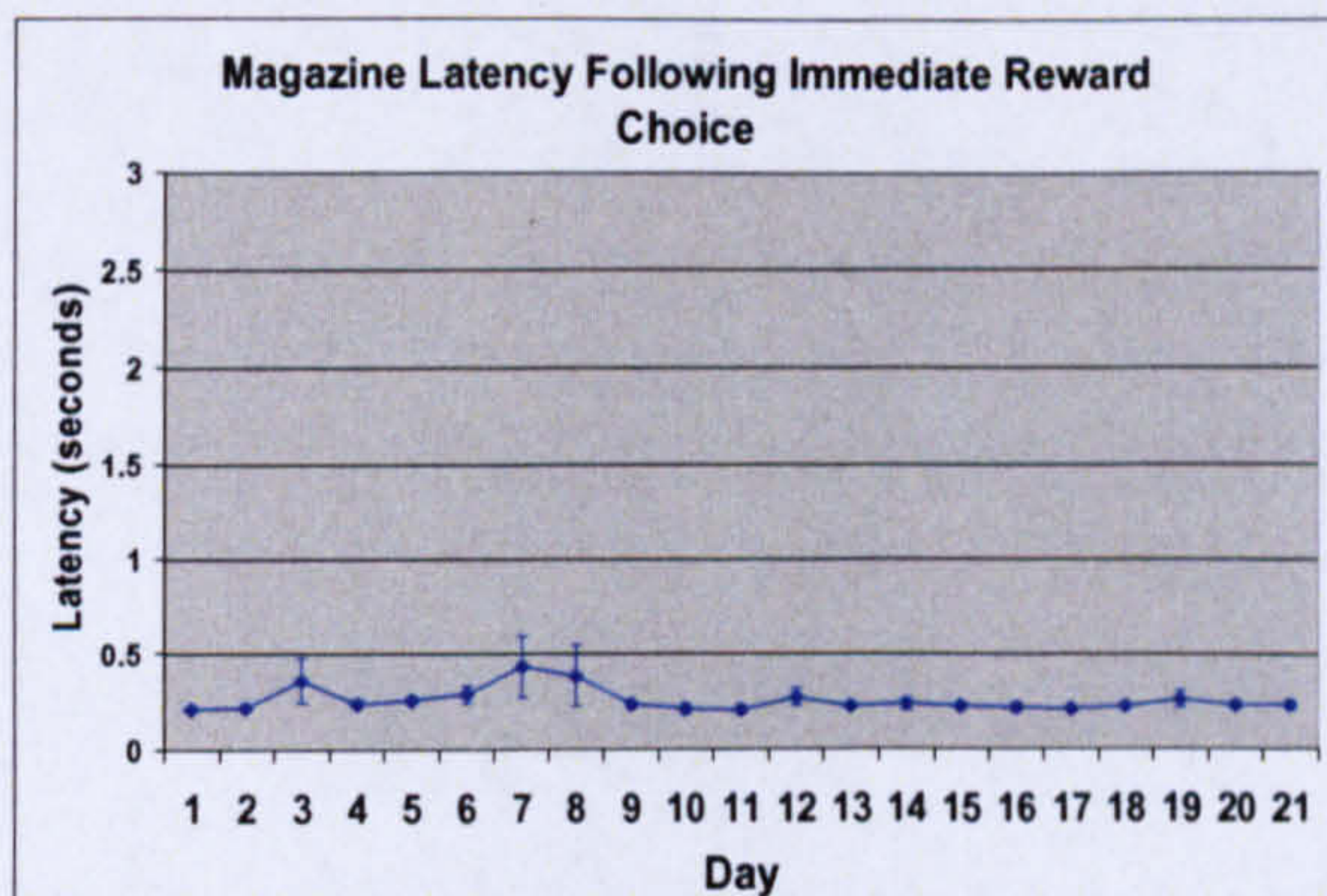
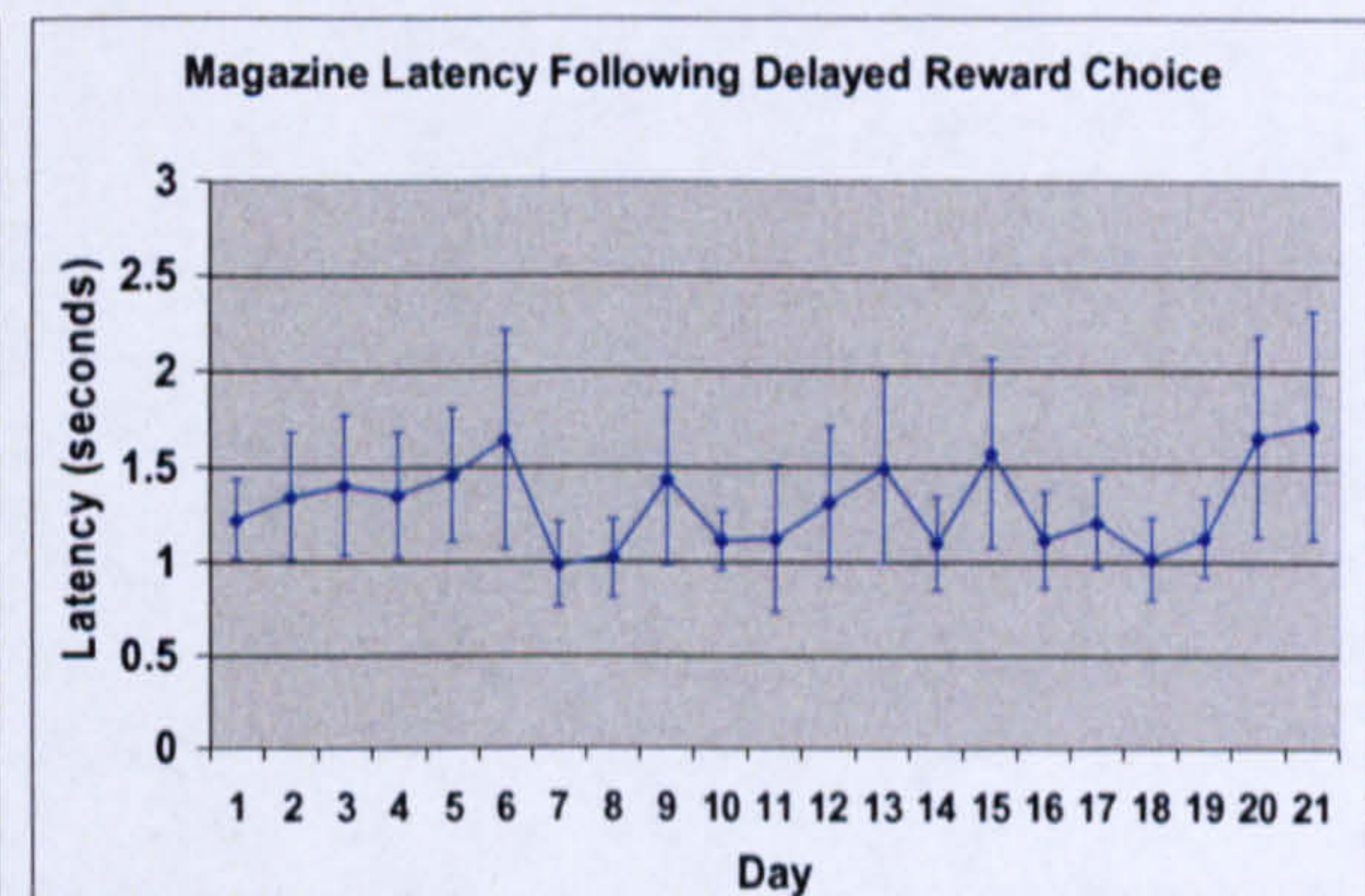
**Fig. 6.4 (a)****Fig. 6.4 (b)****Fig. 6.4 (c)****Fig. 6.4 (d)****Fig. 6.4 (e)**

Fig. 6.4 (a-e): Speed of Responding: the stability of latency in seconds to initiate trials (a), select an immediate reward (b) and select a delayed reward (c). Speed of Responding: the stability of latency in seconds to collect reward following an immediate reward choice (d) and a delayed reward choice (e) across the 21 day period. Each point represents the mean latency in seconds \pm SEM.

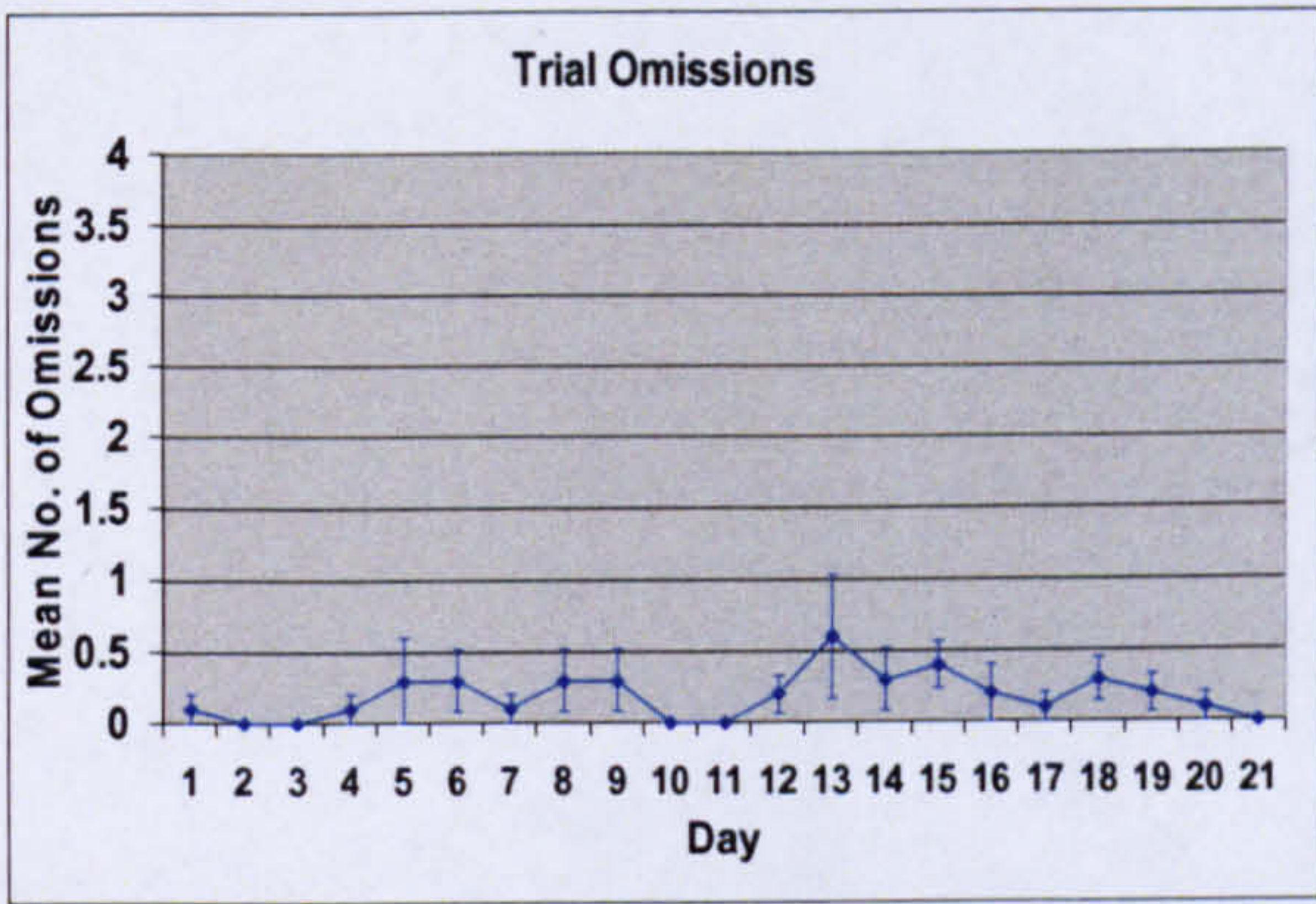


Fig. 6.5 (a)

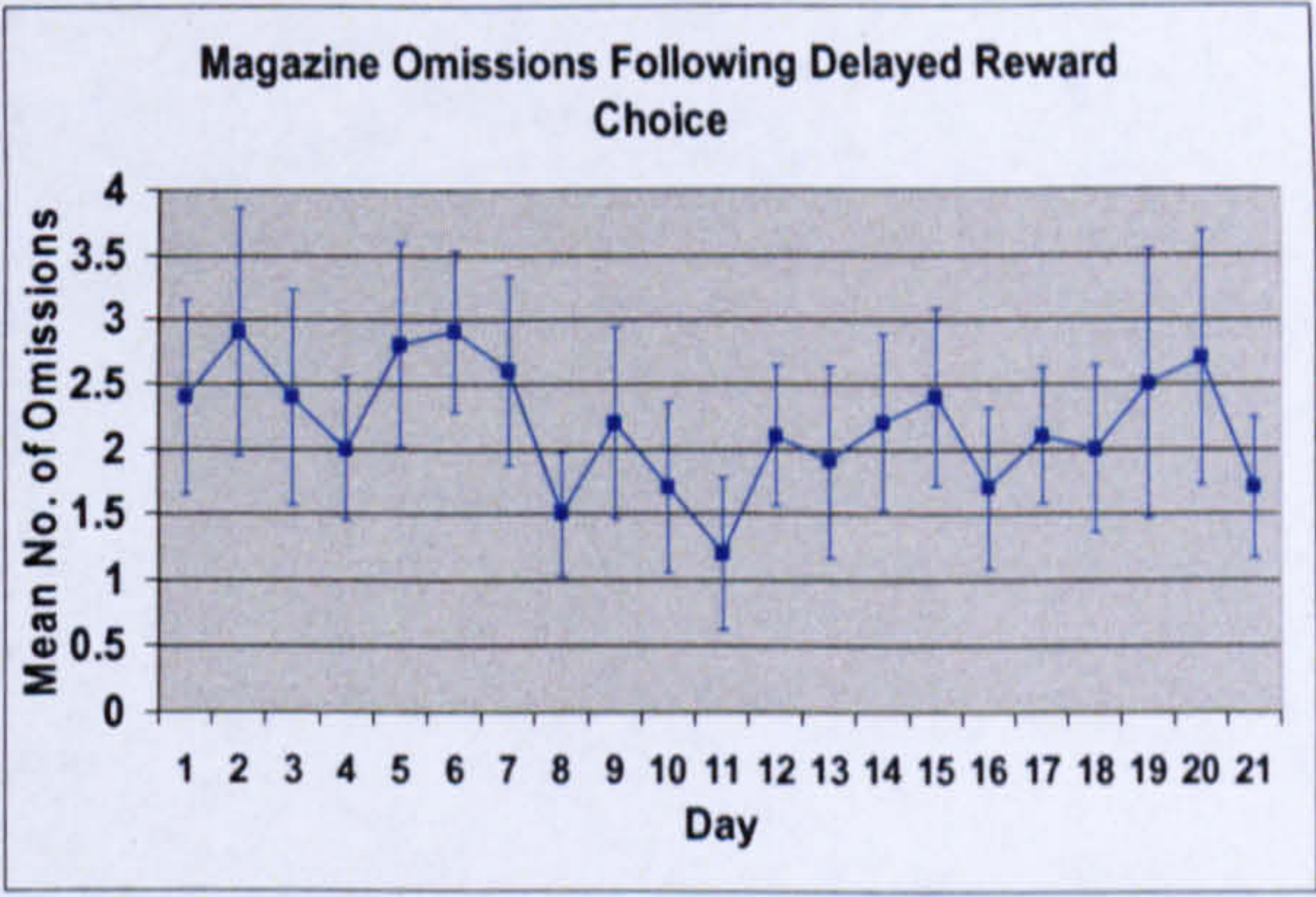


Fig. 6.5 (b)

Fig 6.5: Omissions: stability of frequency trial omissions (a) and magazine omission following a delayed choice (b) across the 21 day period. Each point represents the mean frequency \pm SEM.

6.3.2.4. Individual Variation in Levels of Impulsive Choice

Despite animals receiving identical training in the task, as illustrated in Fig. 6.6, marked individual differences were observed in the rate of discounting of delayed reward. The rate of exponential decay of choice of the larger delayed reward was determined for each animal. The k coefficient obtained for each subject was plotted and is summarised in Table 6.2, in addition to the R^2 values. Subjects 3 ($k = 0.864$) and 8 ($k = 1.309$) were identified as extreme outliers as they displayed significantly greater k values in comparison to the rest of the group (mean $k = 0.198 \pm 0.031$). The R^2 values displayed in Table 6.2 indicate that the exponential discounting function provided a good characterisation of the individual animal data.

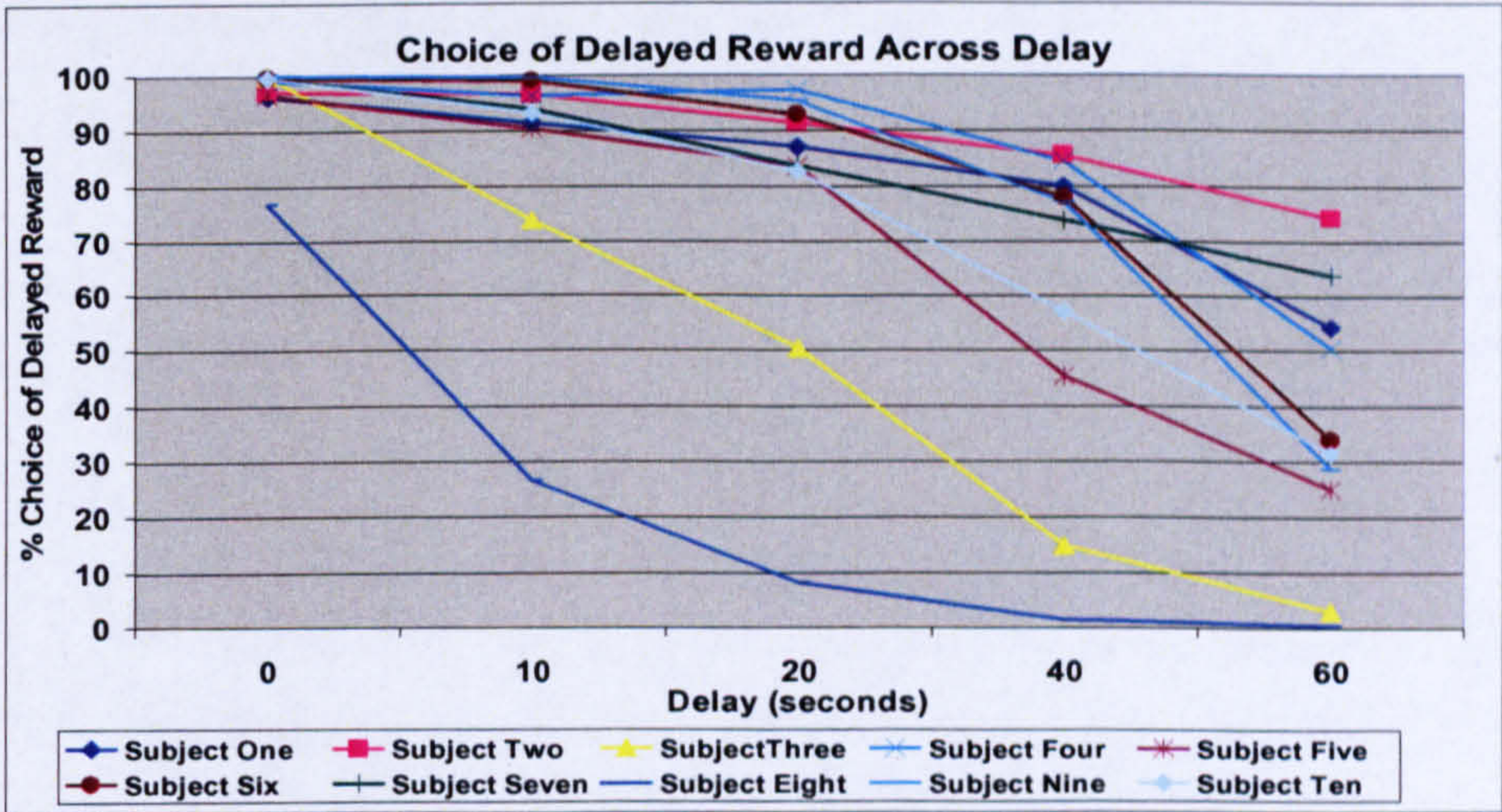


Fig. 6.6: Choice Behaviour: individual variability in the average rate of discounting of delayed reward during the 21 day stability test period. Each point represents the mean percentage choice.

Table 6.2: Individual rat data, indicating steepness of discounting (k) and goodness of fit (R^2)

Subject	k value	R^2 value
1	0.13	0.78
2	0.07	0.88
3	0.86	0.89
4	0.15	0.76
5	0.34	0.86
6	0.24	0.69
7	0.11	0.99
8	1.31	0.98
9	0.28	0.66
10	0.28	0.86
Mean \pm SEM	0.38 \pm 0.13	0.84 \pm 0.04

6.3.3. EXPERIMENT 5B: EXAMINATION OF THE ACUTE EFFECTS OF ALTERATIONS IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

6.3.3.1. DECREASE IN PRIMARY MOTIVATION: Effects of 1 h Prefeeding of Normal Rat Chow

During 1 hr prefeeding of normal rat chow animals consumed an average of 11.72 ± 0.50 g. Following feeding, an average of 12.88 ± 0.71 g of BW was gained, increasing BW on average by 2.54 ± 0.14 %.

6.3.3.1.1. Choice Behaviour

Prefeeding for a 1 hr period prior to operant testing had no significant effect on overall percentage choice of delayed reward in the task in comparison to baseline ($t = 1.527$, $df = 9$, N.S.) (Overall choice of delayed reward: BL- 69.96 ± 6.34 %; following prefeeding -65.04 ± 6.42 %). Analysis of choice by delay furthermore revealed no significant main effect of level of motivation on choice of delayed reward ($F(1,9) = 0.577$, N.S.). A highly significant main effect of delay was indicated ($F(4,36) = 41.652$, $p < 0.001$), with post hoc analysis demonstrating a reduction in choice of delayed reward at 20, 40 and 60 seconds in comparison to choice behaviour during 0 delay trials (all $p < 0.01$). No significant motivation \times delay interaction was observed ($F(4,36) = 0.661$, N.S.) (see Fig. 6.7).

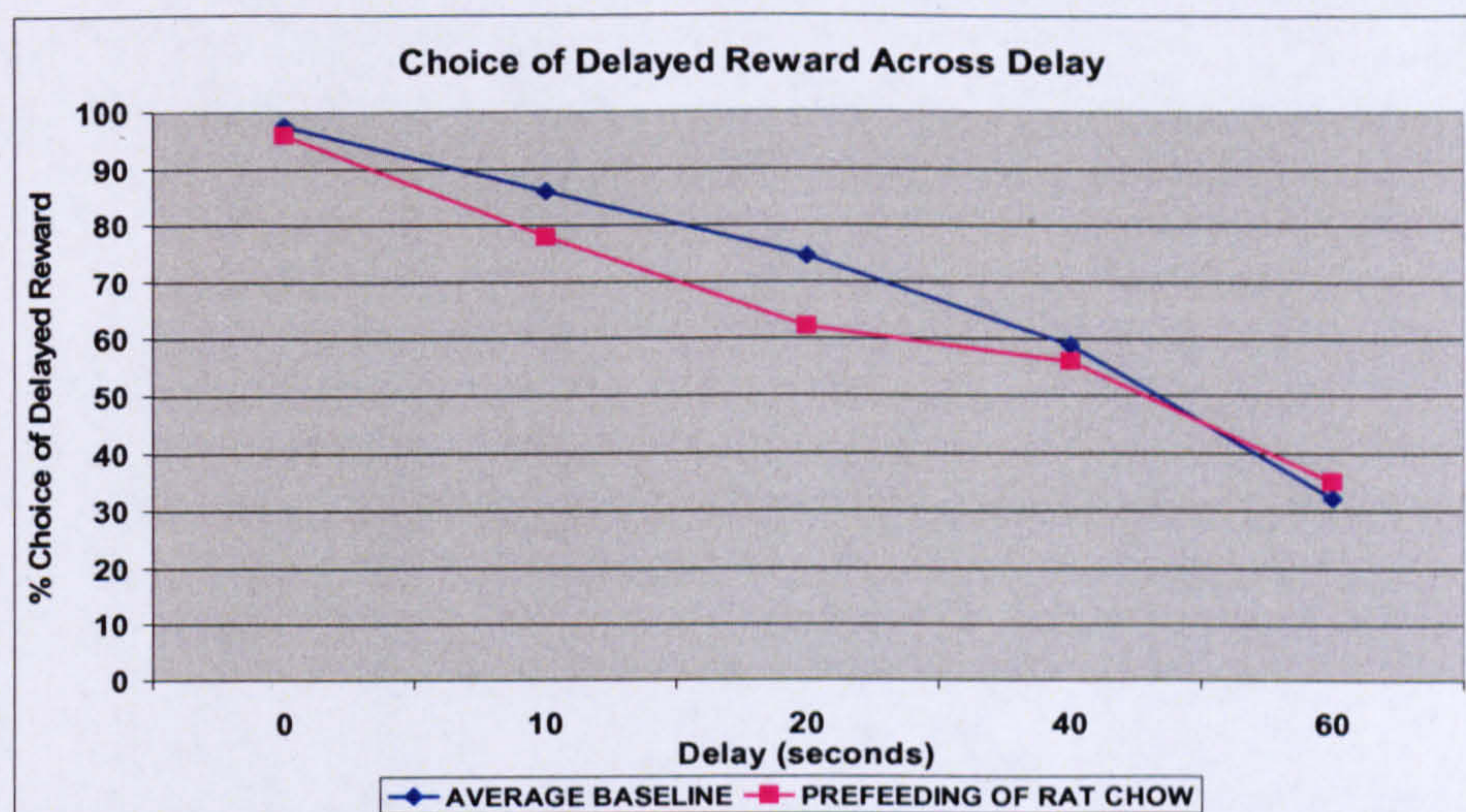


Fig. 6.7: The effects prefeeding of normal rat chow on choice of delayed reward across delay condition. Each point represents the mean percentage \pm SEM.

6.3.3.1.2. Speed of Responding

Table 6.3 summarises the effects of alterations in primary motivation on speed of responding measures in the task, relative to prior average BL performance. Reducing motivation through prefeeding with normal rat chow significantly increased the latency to initiate trials in the task ($t = -4.471$, $df = 9$, $p = 0.001$). The speed with which the immediate reward was selected was slower in comparison to BL ($t = -2.326$, $df = 9$, $p = 0.045$). However, the latency to select the delayed larger reward was not altered by prefeeding ($t = -1.892$, $df = 9$, N.S.).

Following prefeeding, the speed with which the reward was collected following both an immediate and delayed reward choice was found not to differ from BL latency ($t = -1.170$, $df = 9$, N.S.; $t = 1.325$, $df = 9$, N.S.) (see Table 6.3).

6.3.3.1.3. Omissions

Reducing motivation had no significant effect on the frequency of trial omissions ($Z = -1.389$, $N = 10$; N.S.) or failure to collect the reward following a delayed reward choice ($Z = -1.122$, $N = 10$; N.S.). Table 6.4 displays the effects of alterations in motivation on frequency of omissions in the task, in comparison to BL frequency.

6.3.3.2. DECREASE IN PRIMARY MOTIVATION: Effects of 30 Mins Prefeeding of Sucrose Reward Pellets

Animals consumed an average of $12.49 \pm 0.84g$ during the 30 minutes of free access to sucrose reward pellets, increasing BW immediately prior to feeding by $12.32 \pm 1.03g$ - a percentage increase of $2.38 \pm 0.21\%$.

The amount of normal rat chow and sucrose pellets did not differ significantly across prefeeding manipulations ($t = -1.100$, $df = 9$, N.S.). No differences were observed between the

manipulations in the BW gained following each of the feeding sessions, as measured by actual weight gain and percentage increase in weight ($t = 0.550$, $df = 9$, N.S.; $t = 0.766$, $df = 9$, N.S., respectively).

6.3.3.2.1. Choice Behaviour

Despite an observed trend for a decrease in overall choice of delayed reward following prefeeding with sucrose pellets, this effect failed to reach significance ($t = 2.231$, $df = 9$, N.S.) (Overall choice of delayed reward: BL- $73.30 \pm 6.18\%$; following prefeeding- $68.01 \pm 6.66\%$). Choice of behaviour by delay indicated no significant main effect of level of motivation ($F(1,9) = 2.849$, N.S.), although choice behaviour was delay-dependent ($F(4,36) = 35.766$, $p < 0.001$). Post hoc comparisons indicated that the choice of the delayed reward was significantly lower at 40 and 60 seconds relative to the choice during the 0 second delay condition (all $p < 0.01$). Level of motivation was not found to interact with test day ($F(4,36) = 0.791$, N.S.) (see Fig. 6.8).

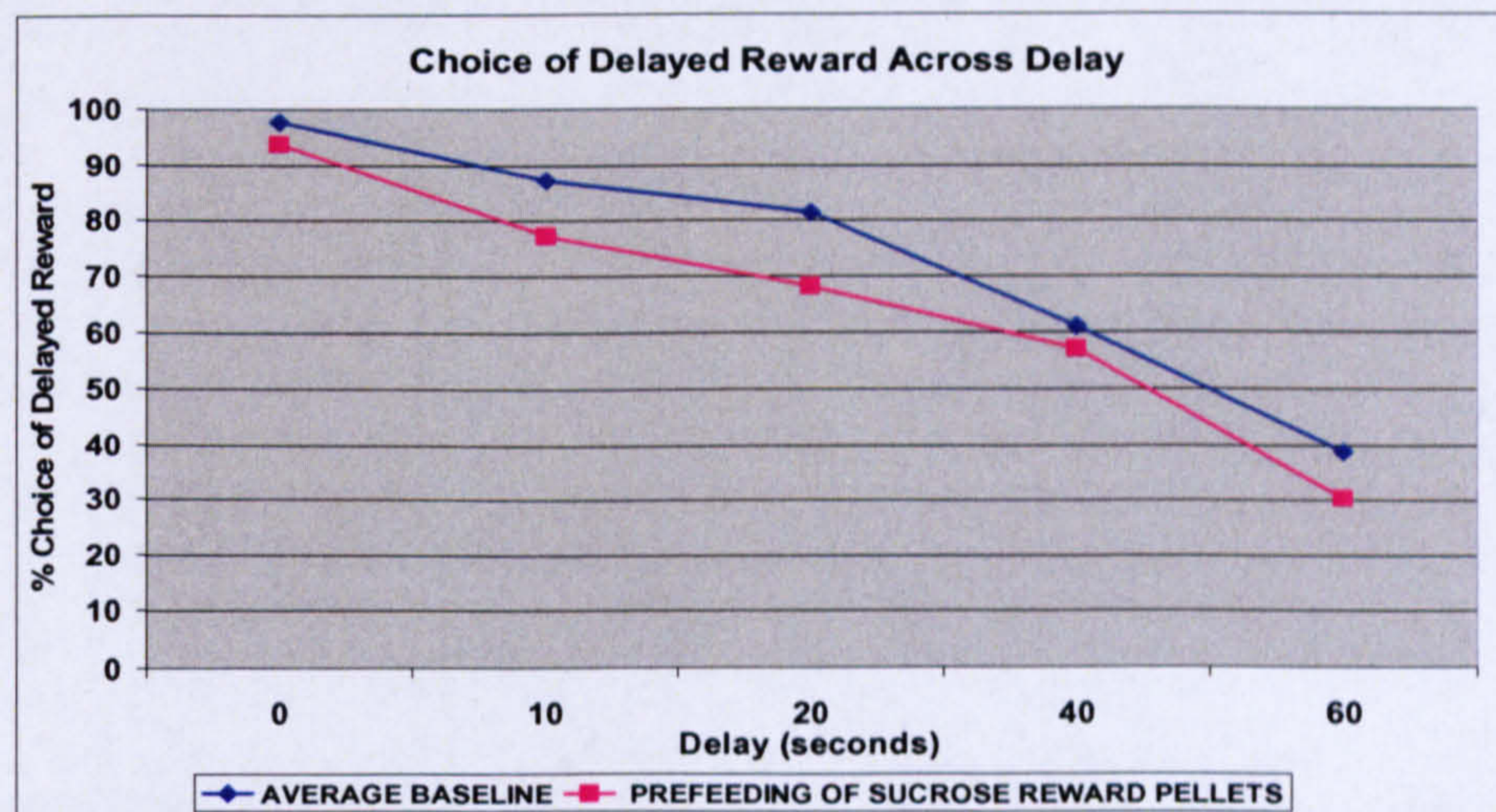


Fig. 6.8: The effects prefeeding of sucrose reward pellets on choice of delayed reward across delay condition. Each point represents the mean percentage \pm SEM.

6.3.3.2.2. Speed of Responding

Latency to initiate trials became significantly slower following prefeeding with sucrose reward pellets ($t = -2.998$, $df = 9$, $p = 0.015$) (see Table 6.3). The speed with which both the immediate and delayed reward were selected did not differ from BL latency ($t = -1.589$, $df = 9$, N.S.; $t = 1.476$, $df = 9$, N.S., respectively).

After prefeeding with sucrose reward pellets, the magazine latencies for the immediate reward choice remained comparable to BL ($t = 0.565$, $df = 9$, N.S.); however, there was a slowing in the speed with which the delayed reward was collected ($t = -3.229$, $df = 9$, $p = 0.009$) (Table 6.3).

6.3.3.2.3. Omissions

Prefeeding with sucrose reward pellets prior to operant testing had no significant effect on the failure to initiate trials or collect the reward following a delayed reward choice ($Z = -1.219$, $N = 10$; N.S.; $Z = -1.130$, $N = 10$; N.S.) (see Table 6.4).

6.3.3.3. INCREASE IN PRIMARY MOTIVATION: Effect of Reducing Food Consumption the Day Prior to Testing

The reduction of food allowance by 50% on the day prior to testing led to a loss of an average of 4.52 ± 0.72 g of BW and percentage decrease in weight of $1.01 \pm 0.002\%$.

6.3.3.3.1. Choice Behaviour

Increasing motivation for food reward led to a significant increase in overall percentage choice of the delayed larger reward ($t = -2.507$, $df = 9$, $p = 0.033$) (Overall choice of delayed reward: BL- $70.06 \pm 7.18\%$; following reduction of daily food intake $73.06 \pm 8.07\%$). Analysis of choice by delay further supported the increased preference for the delayed reward ($F(4,36) = 8.253$, $p = 0.018$). A main effect of delay was also revealed ($F(4,36) = 32.826$, $p < 0.001$), indicating that the choice of the delayed larger reward decreased with increasing delays, reaching significance at the 40 and 60 second delay conditions in comparison to choice during 0 second delay trials (all $p < 0.001$). The effect of increasing motivation on choice behaviour was delay dependent, as indicated by the significant motivation x delay interaction ($F(4,36) = 5.688$, $p = 0.001$). To examine this interaction further, a series of repeated measures t-tests compared choice behaviour at each delay (see Fig. 6.9). Following the increase in motivation, the choice of the delayed reward was significantly increased during the 10 second delay condition in comparison to choice at this delay under BL conditions ($t = -5.577$, $df = 9$, $p < 0.001$). Choice of delayed reward across all remaining delay conditions remained comparable to BL choice (all $t \geq 0.210$, $df = 9$, N.S.).

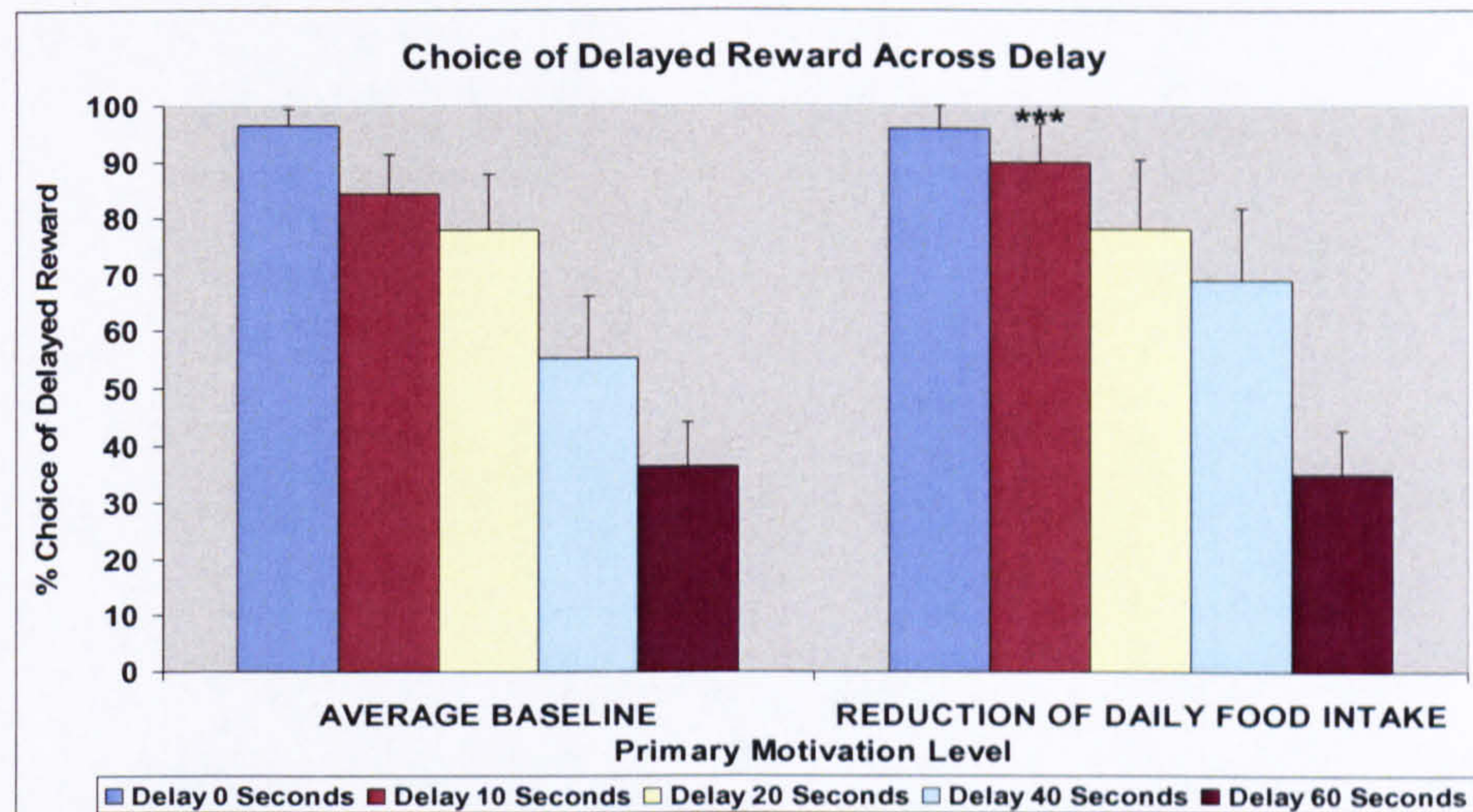


Fig. 6.9: Simple effects analysis of motivation*delay interaction: The effect of reducing food intake the day prior to testing on percentage choice of delayed reward across delay condition. Comparison of choice at each delay. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to baseline.

Independent analysis of the main effect of delay during BL and following an increase in motivation for food reward, indicated choice behaviour was delay-sensitive during both conditions ($F(4, 36) = 40.115$, $p < 0.001$; $F(4, 36) = 23.608$, $p < 0.001$). As illustrated in Fig. 6.10, during BL choice of delayed reward was significantly lower at 10, 40 and 60 seconds delay in comparison to choice behaviour when no delays were present during trials (all $p < 0.01$). However, after one day of reduced daily food allowance, choice of delayed reward was only significantly reduced at the highest 60 second delay condition relative to choice during the 0 second delay condition ($p < 0.001$).

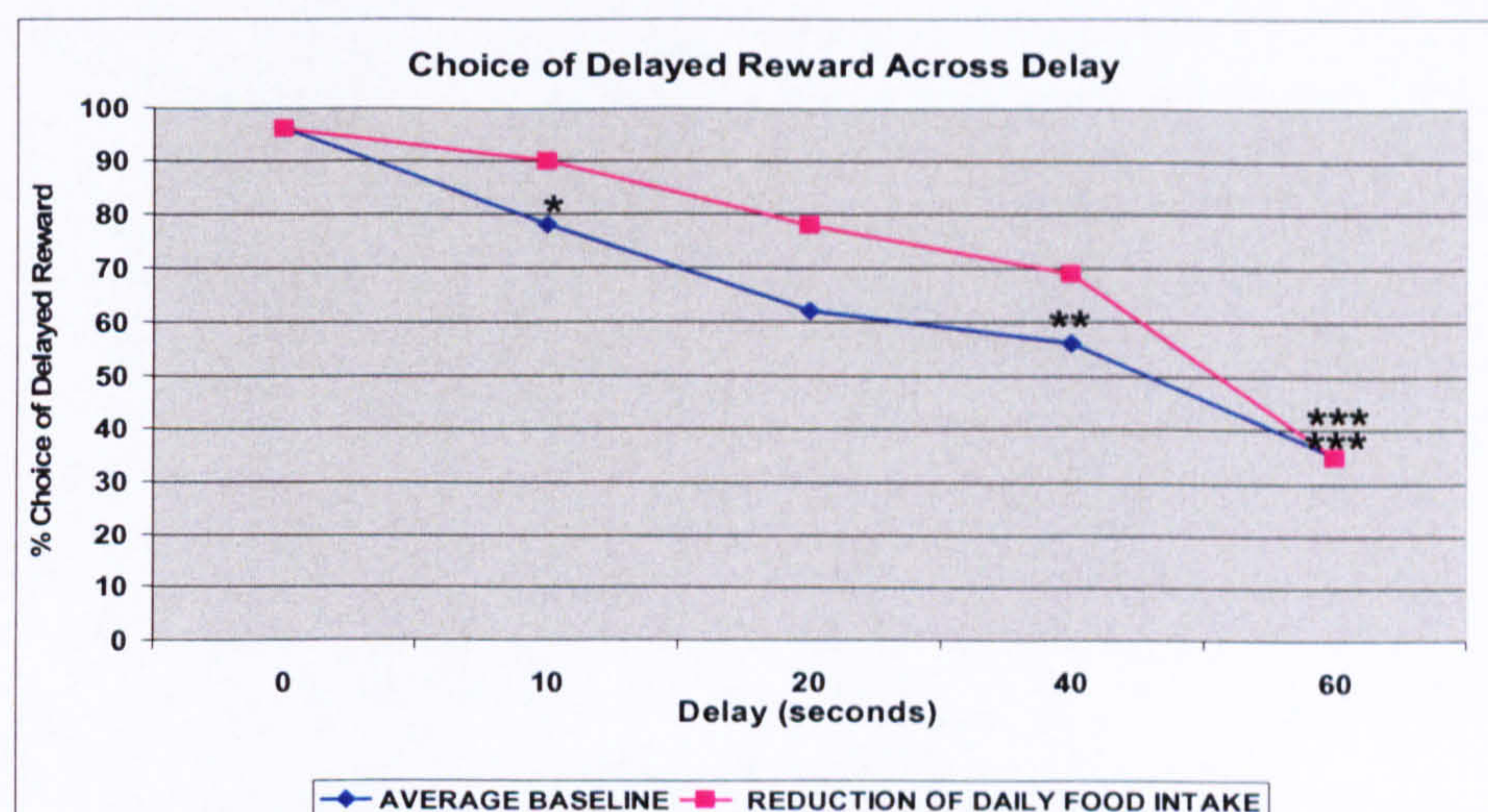


Fig. 6.10: Simple effects analysis of motivation*delay interaction: The effect of reducing food intake the day prior to testing on rate of discounting of delayed reward under each condition. Each point represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

6.3.3.3.2. Speed of Responding

Increasing levels of food deprivation prior to testing had no significant effect on the latency to initiate trials ($t = -1.327$, $df = 9$, N.S.), or the speed with which animals selected an immediate or delayed reward ($t = -0.260$, $df = 9$, N.S., $t = -0.740$, $df = 9$, N.S., respectively) (see Table 6.3).

Magazine latency following an immediate reward choice furthermore did not differ from BL ($t = -1.189$, $df = 9$, N.S.). In contrast, the speed with which animals collected reward following a delayed reward choice became significantly faster ($t = 1.905$, $df = 9$, $p = 0.045$) (see Table 6.3).

6.3.3.2.3. Omissions

Increasing levels of food deprivation significantly decreased the frequency of failures to initiate trials during task performance ($Z = -2.060$, $N = 10$, $p = 0.039$). Magazine omissions following a delayed reward choice did not differ significantly from BL ($Z = 0.001$, $N = 10$, N.S.) (see Table 6.4).

Table 6.3: The effects of acute alterations in motivation for food reward on speed of responding in the delayed reinforcement task

Behavioural Measure	Average BL Prior to Prefeeding of Rat Chow	Sated Following Prefeeding of Rat Chow	Average BL Prior to Prefeeding of Sucrose Pellets	Sated Following Prefeeding of Sucrose Pellets	Average BL Prior to Reduction of Daily Food Intake	Increased Deprivation Following Reduction of Daily Food Intake
Initiation Latency	1.13 ± 0.07	1.76 ± 0.13**	1.30 ± 0.08	1.84 ± 0.18*	1.18 ± 0.07	1.26 ± 0.11
Immediate Response Latency	0.66 ± 0.04	0.88 ± 0.12*	0.66 ± 0.04	0.72 ± 0.03	0.65 ± 0.04	0.65 ± 0.06
Delayed Response Latency	0.67 ± 0.04	0.74 ± 0.06	0.68 ± 0.03	0.72 ± 0.04	0.66 ± 0.03	0.68 ± 0.05
Immediate Magazine Latency	0.22 ± 0.02	0.25 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.21 ± 0.02
Delayed Magazine Latency	1.50 ± 0.54	0.96 ± 0.23	1.74 ± 0.75	2.43 ± 1.76**	1.19 ± 0.32	0.91 ± 0.19*

Table 6.3: Each value represents the mean latency (seconds) ± SEM. . *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to baseline.

Table 6.4: The effects of acute alterations in motivation for food reward on omissions in the delayed reinforcement task

Behavioural Measure	Average BL Prior to Prefeeding of Rat Chow	Sated Following Prefeeding of Rat Chow	Average BL Prior to Prefeeding of Sucrose Pellets	Sated Following Prefeeding of Sucrose Pellets	Average BL Prior to Reduction of Daily Food Intake	Increased Deprivation Following Reduction of Daily Food Intake
Trial Omissions	0.26 ± 0.13	1.50 ± 0.86	0.24 ± 0.17	2.80 ± 1.81	0.32 ± 0.20	0.00 ± 0.00
Magazine Omission Following Delayed Choice	1.68 ± 0.55	1.30 ± 0.88	2.42 ± 1.09	2.60 ± 1.96	1.62 ± 1.80	0.54 ± 0.63**

Table 6.4: Each value represents the mean frequency ± SEM. . *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to baseline.

6.3.4. EXPERIMENT 5C: EXAMINATION OF THE EFFECTS OF CHRONIC ALTERATION IN PRIMARY MOTIVATION ON PERFORMANCE IN THE DELAYED REWARD TASK

Maintaining animals on a 20% greater daily food allowance for a one week period resulted in a significant greater BW gain in comparison to the prior BL week under the normal feeding regime (absolute BW gain, $t = -11.658$, $df = 9$, $p < 0.001$; percentage increase in BW, $Z = -2.803$, $p = 0.005$, respectively) (See Table 6.5).

Table 6.5: Long-term decrease in primary motivation on BW change

Body Weight Change	Seven Day Baseline	Long Term Decrease in Motivation
Absolute BW gain (g)	+1.16 ± 0.83	+16.36 ± 1.07***
Percentage Change in BW	+0.22 ± 0.15	+3.07 ± 0.18**

Table 6.5: Each value represents the mean ± SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to baseline.

6.3.4.1. EFFECTS OF A PROLONGED DECREASE IN PRIMARY MOTIVATION

6.3.4.1.1. Choice Behaviour

Increasing the daily food allowance by 20% had no effect on overall choice of delayed reward in the task ($F(7,63) = 2.462$, N.S.) (see Fig. 6.11). Analysis of the choice of reward by delay revealed no significant main effect of level of motivation ($F(7, 63) = 1.816$, N.S.). Evidence that choice behaviour was sensitive to delay was however shown, indicated by a main effect of delay ($F(4,36) = 41.903$, $p < 0.001$). Further analysis revealed that choice of delayed reward was significantly reduced at 40 and 60 seconds relative to choice during 0 second delay trials (all $p < 0.001$). No significant delay x day interaction on choice behaviour was observed ($F(28, 252) = 0.858$, N.S.) (see Fig. 6.12).

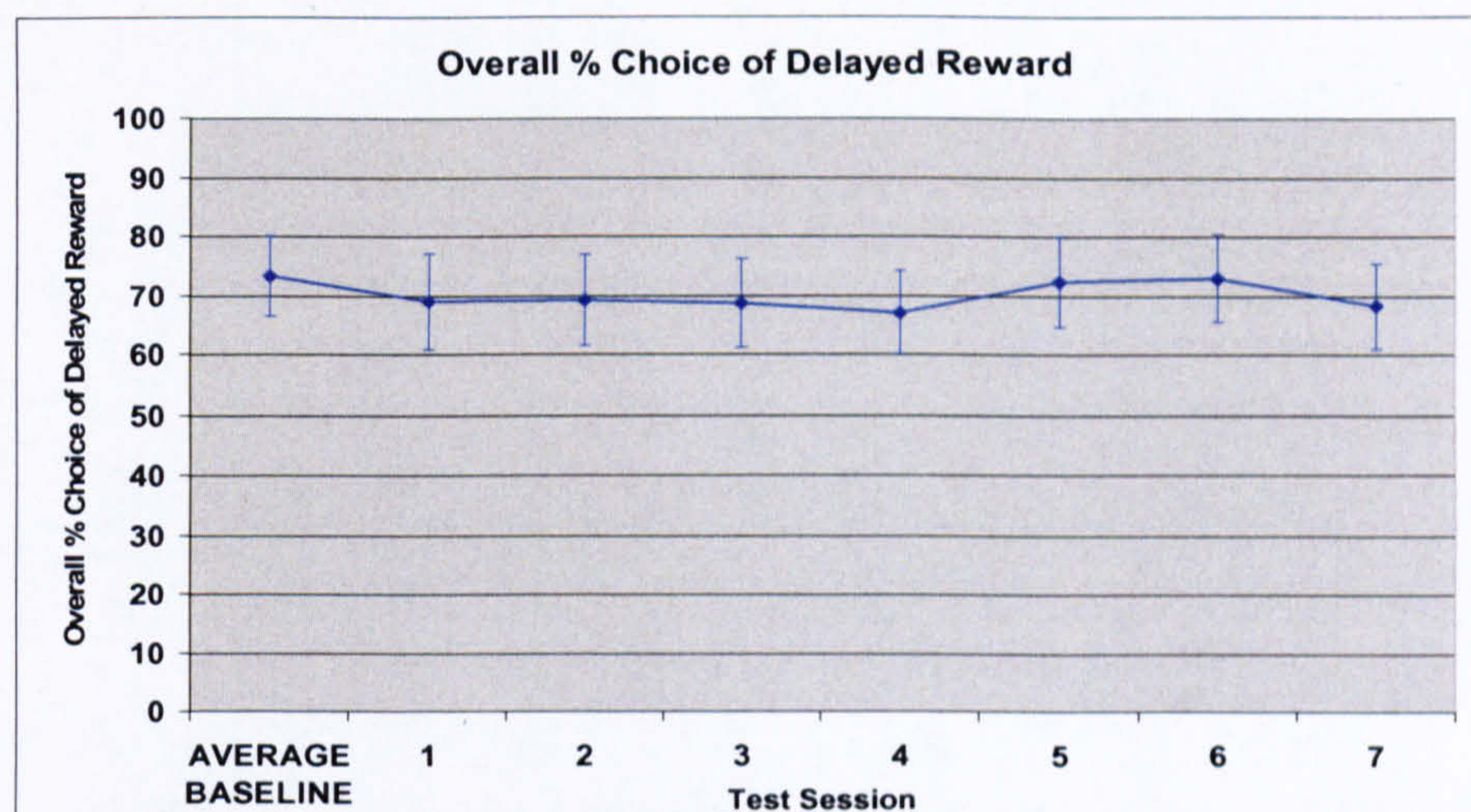


Fig. 6.11: The effect of prolonged reduction in primary motivation on overall percentage choice of delayed reward. Each point represents the mean percentage \pm SEM.

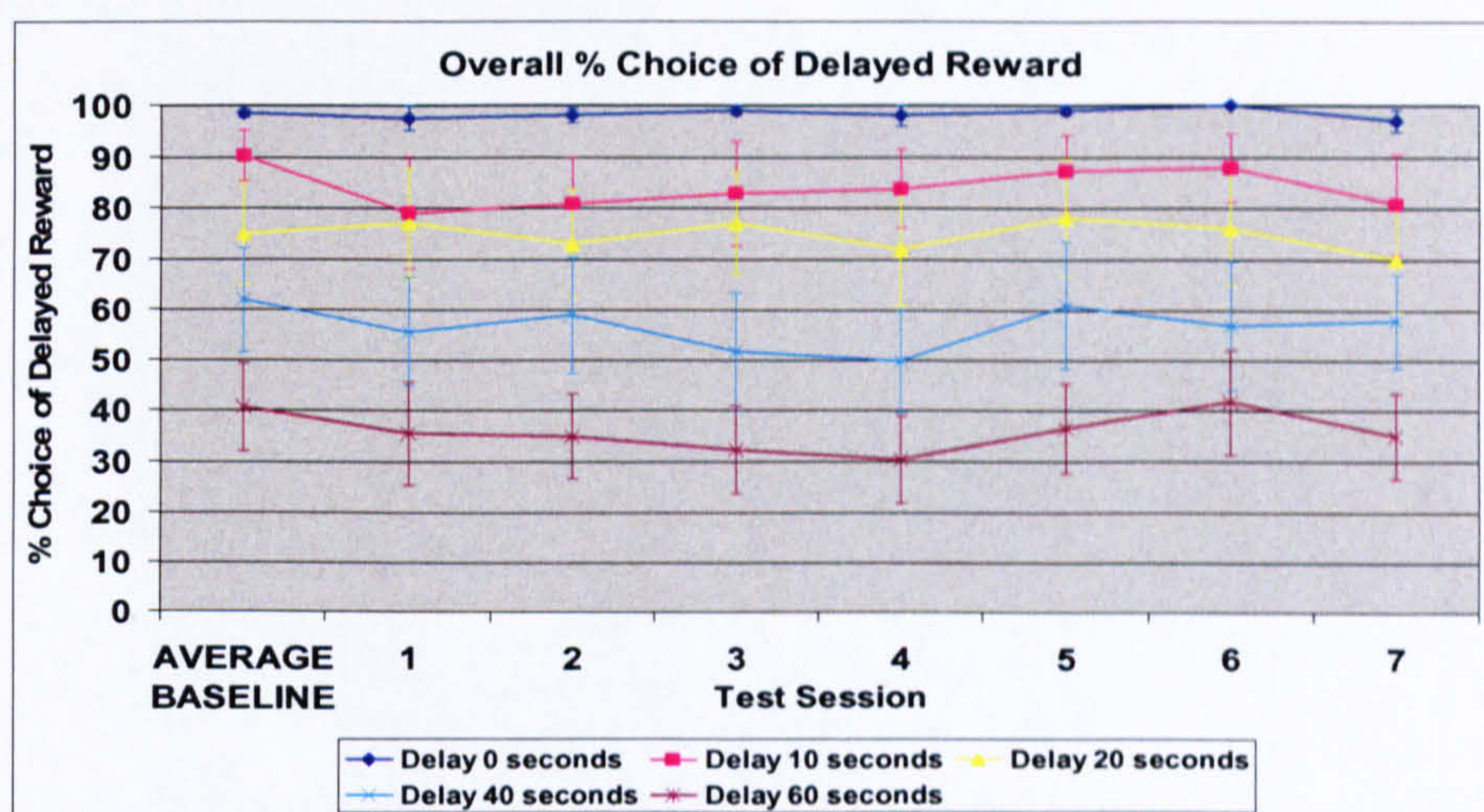


Fig. 6.12: The effect of prolonged reduction in primary motivation on percentage choice of delayed reward across delay condition. Each point represents the mean percentage \pm SEM.

6.3.4.1.2. Speed of Responding

No main effect of day was revealed on the latency to initiate trials ($F(7,63) = 0.698$, N.S.). Increasing daily food allowance across seven days moreover had no significant effect on speed with which animals selected the delayed and immediate reward ($F(1.507, 13.559) = 1.097$, N.S.; $F(2.359, 21.223) = 0.403$, N.S., respectively), or the latency at which the reward was collected ($F(2.100, 18.903) = 0.188$, N.S.; $F(1.927, 17.342) = 0.374$, N.S., respectively). Table 6.7 summarises the effects of long term alterations of primary motivation for food reward on speed of responding parameters in the task.

6.3.4.1.3. Omissions

No significant main effect of day was observed on the failure to initiate trials or to collect reward following a delayed reward choice ($X^2 = 10.637$, $df = 7$, N.S.; $X^2 = 2.059$, $df = 7$, N.S., respectively) (see Table 6.8).

6.3.4.2. EFFECTS OF A PROLONGED INCREASE IN PRIMARY MOTIVATION

Reducing daily food intake by 20% for a one week period resulted in significant loss of BW in comparison to BL week, prior to the manipulation under the normal feeding regime (absolute BW gain, $t = -8.130$, $df = 9$, $p < 0.001$; percentage increase in BW, $Z = -2.803$, $p = 0.005$, respectively) (See Table 6.6).

Table 6.6: Long-term increase in primary motivation on BW change

Body Weight Change	Seven Day Baseline	Long Term Increase in Motivation
Absolute BW gain (g)	+1.22 \pm 0.93	-11.50 \pm 0.88**
Percentage Change in BW	+0.22 \pm 0.17	-2.15 \pm 0.17**

Table 6.6: Each value represents the mean \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to baseline.

6.3.4.2.1. Choice Behaviour

Figure 6.13 illustrates the overall choice of delayed reward during the seven days where motivation for food reward was increased. Analysis demonstrated a significant main effect of day during this period ($F(7,63) = 6.210$, $p < 0.001$). Post hoc comparisons revealed that the choice of the delayed reward was significantly increased on the final 7th day of reduced food intake in comparison to choice preference during BL. Analysis of choice by delay also revealed a significant main effect of day ($F(4, 36) = 7.179$, $p < 0.001$), indicating once again the significant increased preference of delayed reward on the final day of the motivational manipulation. A significant main effect of delay was also revealed ($F(4, 36) = 27.312$, $p < 0.001$), with selection of the larger, delayed reward being significantly decreased at 40 and 60 second delay conditions in comparison to 0 second delay trials (all $p < 0.05$). The effect of a prolonged increase in deprivation on decreasing choice of delayed reward was not however delay dependent, as indicated by the non-significant motivation \times delay interaction ($F(28, 252) = 1.019$, N.S.).

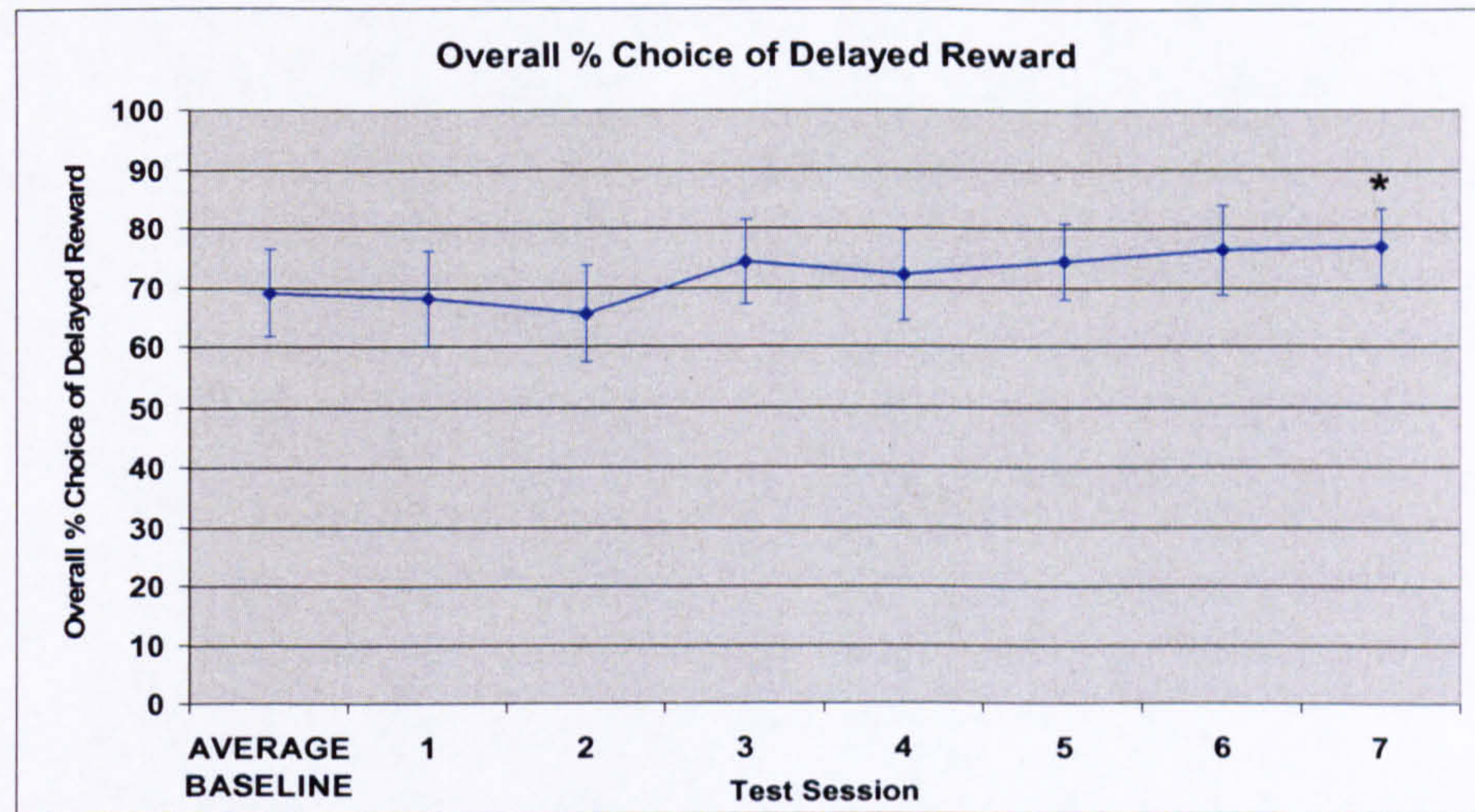


Fig. 6.13: The effect of prolonged increase in primary motivation on overall percentage choice of delayed reward. Each point represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to baseline.

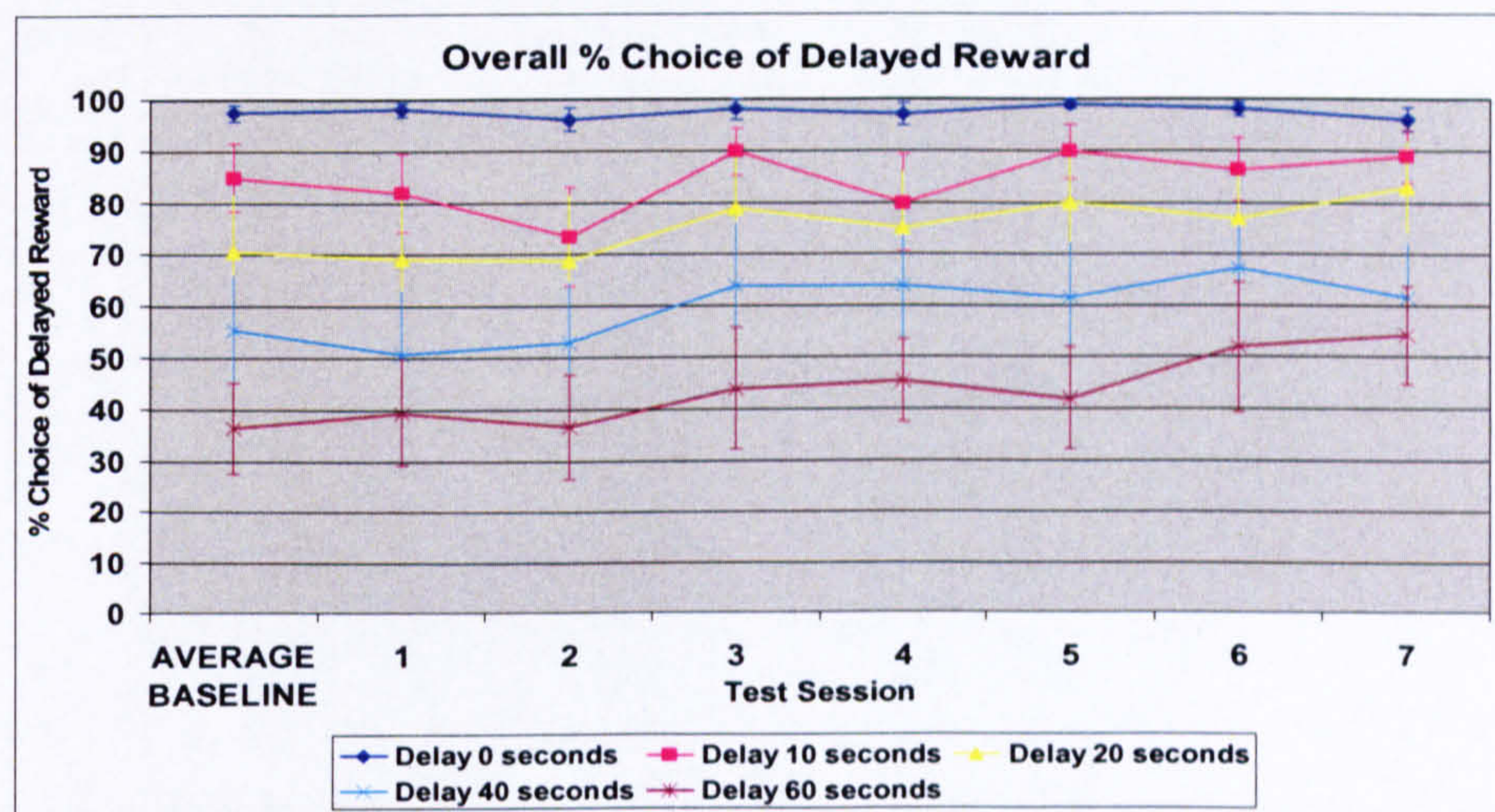


Fig. 6.14: The effect of prolonged increase in primary motivation on percentage choice of delayed reward across delay condition. Each point represents the mean percentage \pm SEM.

6.3.4.2.2. Speed of Responding

The prolonged decrease in daily food allowance had no significant main effect on the latency to initiate trials ($F(7, 63) = 1.075$, N.S.), or the speed with which both the immediate and delayed reward were selected in the task ($F(1.897, 17.077) = 0.686$, N.S.; $F(1.994, 17.943) = 0.935$, N.S., respectively). Latencies to collect reward following both a delayed and immediate reward choice also remained unchanged from baseline ($F(7, 63) = 1.882$, N.S.; $F(1.602, 14.419) = 0.223$, N.S.) (see Table 6.9).

6.3.4.2.3. Omissions

The frequency of initiation of trial omissions and failure to collect delayed reward did not differ from baseline performance ($X^2 = 1.928$, $df = 7$, N.S.; $X^2 = 10.742$, $df = 7$, N.S., respectively) (see Table 6.10).

Table 6.7: Speed of responding in the delayed reinforcement task during prolonged decrease in deprivation for food reward

Behavioural Measure	Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Initiation Latency	1.23 ± 0.11	1.26 ± 1.14	1.24 ± 0.12	1.38 ± 0.19	1.34 ± 0.14	1.33 ± 0.14	1.34 ± 0.15	1.33 ± 0.13
Immediate Response Latency	0.69 ± 0.06	0.78 ± 0.06	0.70 ± 0.04	0.72 ± 0.05	0.83 ± 0.16	0.66 ± 0.04	0.67 ± 0.05	0.69 ± 0.05
Delayed Response Latency	0.77 ± 0.06	0.77 ± 0.12	0.80 ± 0.10	0.78 ± 0.09	0.77 ± 0.08	0.78 ± 0.08	0.77 ± 0.08	0.69 ± 0.05
Immediate Magazine Latency	0.22 ± 0.03	0.23 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.23 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
Delayed Magazine Latency	2.47 ± 1.27	2.39 ± 1.47	2.05 ± 1.04	2.58 ± 1.81	2.41 ± 1.30	2.03 ± 1.38	2.56 ± 1.60	2.43 ± 1.72

Table 6.7: Each value represents the mean latency (seconds) ± SEM.

Table 6.8: Omissions in the delayed reinforcement task during prolonged decrease in deprivation for food reward

Behavioural Measure	Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Trial Omissions	0.32 ± 0.10	0.30 ± 0.21	0.10 ± 0.10	0.60 ± 0.27	0.90 ± 0.23	1.10 ± 0.40	0.60 ± 0.34	1.33 ± 0.13
Magazine Omission Following Delayed Choice	2.62 ± 1.81	3.10 ± 2.45	2.60 ± 1.73	2.70 ± 2.04	2.50 ± 1.86	2.80 ± 2.26	3.50 ± 2.74	0.69 ± 0.05

Table 6.8: Each value represents the mean frequency ± SEM.

Table 6.9: Speed of responding in the delayed reinforcement task during prolonged increase in deprivation for food reward

Behavioural Measure	Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Initiation Latency	1.39 ± 0.09	1.42 ± 0.13	1.47 ± 0.12	1.41 ± 0.14	1.44 ± 0.12	1.57 ± 0.16	1.52 ± 0.23	1.26 ± 0.13
Immediate Response Latency	0.69 ± 0.05	0.69 ± 0.05	0.77 ± 0.07	0.69 ± 0.05	0.74 ± 0.07	0.68 ± 0.06	0.78 ± 0.11	0.68 ± 0.05
Delayed Response Latency	0.70 ± 0.04	0.72 ± 0.04	0.73 ± 0.05	0.78 ± 0.07	0.72 ± 0.04	0.74 ± 0.05	0.74 ± 0.05	0.74 ± 0.06
Immediate Magazine Latency	0.22 ± 0.03	0.20 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.25 ± 0.03	0.22 ± 0.03	0.21 ± 0.03	0.21 ± 0.03
Delayed Magazine Latency	2.05 ± 1.13	1.62 ± 1.07	1.78 ± 1.08	2.17 ± 1.10	2.15 ± 1.53	1.88 ± 1.04	2.20 ± 0.93	1.82 ± 0.90

Table 6.9: Each value represents the mean latency (seconds) ± SEM.

Table 6.10: Omissions in the delayed reinforcement task during prolonged increase in deprivation for food reward

Behavioural Measure	Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Trial Omissions	0.66 ± 0.20	1.00 ± 0.54	1.30 ± 0.70	0.90 ± 0.50	1.10 ± 0.48	1.70 ± 0.86	2.00 ± 1.58	0.60 ± 0.31
Magazine Omission Following Delayed Choice	2.49 ± 1.92	2.30 ± 1.99	2.50 ± 2.07	2.90 ± 2.16	1.90 ± 1.90	2.80 ± 1.82	2.60 ± 1.58	2.50 ± 1.95

Table 6.10: Each value represents the mean frequency ± SEM.

6.3.5. EXPERIMENT 5D: EXAMINATION OF THE REDUCTION OF DELAYS TO THE DELIVERY OF REWARD ON PERFORMANCE IN THE DELAYED REWARD TASK

6.3.5.1. Choice Behaviour

Reduction of delays during the task had a profound effect on choice behaviour, increasing significantly overall choice of the delayed larger reward in the task ($t = -6.880$, $df = 9$, $p = 0.001$) (Overall choice of delayed reward: BL- $71.53 \pm 6.76\%$; Following reduction of delays- $83.30 \pm 6.86\%$).

Assessment of choice behaviour by delay, revealed that choice behaviour was delay-dependent during both BL and the delay manipulation, as indicated by the significant main effects of delay ($F(4,36) = 34.582$, $p < 0.001$; $F(4,36) = 5.849$, $p = 0.010$, respectively). Post hoc analysis revealed that, whilst during BL choice of the delayed reward differed significantly from 0 delay trials at 40 and 60 delay conditions (all $p < 0.001$), following the reduction of delay to the delivery of the larger reward no significant differences were observed between choice behaviour across delays (all $p > 0.05$) (see Fig 6.15).

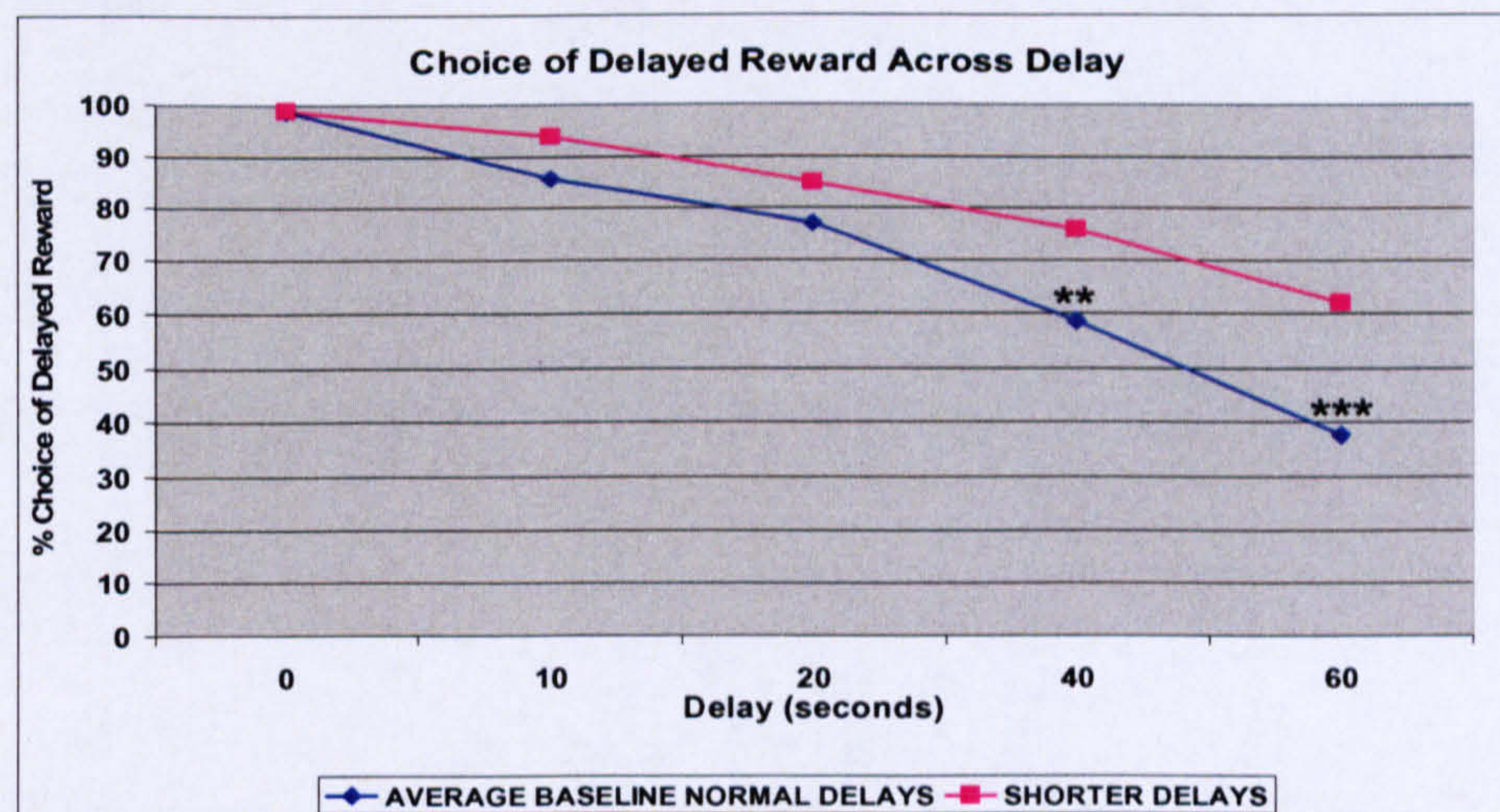


Fig. 6.15: Simple effects analysis of delay length*delay interaction: The effect of reduced task delays on rate of discounting of delayed reward under each condition. Each point represents the mean percentage \pm SEM. *, $p < 0.05$. **, $p < 0.01$, ***, $p < 0.001$ (Bonferonni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

6.3.5.2. Speed of Responding

Reducing delays in the task had no significant effect on the latency to initiate trials ($t = 1.592$, $df = 9$, N.S), or the speed with which animals selected a delayed and immediate choice ($t = 0.592$, $df = 9$, N.S.; $t = 1.333$, $df = 9$, N.S., respectively).

No differences in comparison to BL were indicated on the speed with which reward was

collected following either a delayed or immediate reward choice in the task ($t = 0.097$, $df = 9$, N.S.; $t = -1.711$, $df = 9$, N.S., respectively). See table 6.11 for speed of responding parameters during the delay manipulation.

6.3.5.3. Omissions

The frequency of failures to initiate trials did not differ significantly from BL performance ($Z = -0.316$, $N = 10$, N.S.). However, the number of magazine omissions following the selection of the delayed reward increased significantly when the shorter delays were tested ($Z = -2.103$, $N = 10$, $p = 0.035$) (see Table 6.12).

Table 6.11: Speed of responding in the delayed reinforcement task following reduction in task delays

Behavioural Measure	Average BL Normal Task Delays	Shorter Task Delays
Initiation Latency	1.21 ± 0.05	1.06 ± 0.13
Immediate Response Latency	0.67 ± 0.04	0.69 ± 0.05
Delayed Response Latency	0.72 ± 0.05	0.68 ± 0.04
Immediate Magazine Latency	0.22 ± 0.03	0.24 ± 0.03
Delayed Magazine Latency	1.60 ± 0.73	2.76 ± 1.07

Table 6.11: Each value represents the mean latency (seconds) ± SEM.

Table 6.12: Omissions in the delayed reinforcement task following reduction in task delays.

Behavioural Measure	Average BL Normal Task Delays	Shorter Task Delays
Trial Omissions	0.24 ± 0.16	0.20 ± 0.13
Magazine Omission Following Delayed Choice	1.70 ± 0.97	4.10 ± 1.67*

Table 6.12: Each value represents the mean frequency ± SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to baseline.

6.4. DISCUSSION

The delayed reward task is a paradigm utilised extensively in preclinical research to assess levels of impulsive choice in rodents (e.g. Evenden and Ryan, 1996; Cardinal et al., 2000; Isles et al., 2003; Paine et al., 2003; Winstanley et al., 2003a; Uslaner and Robinson, 2006). The studies reported in the present chapter were conducted to assess the appropriateness of the task for longer term drug studies and to assess the effects of manipulations of primary motivation which are expected to occur following nicotine administration.

The present study replicated findings reported in previous research using this behavioural paradigm (Evenden and Ryan, 1996; Cardinal et al., 2000). As training progressed, animals' choice behaviour became contingent upon the delay to the delivery of the larger reward. Rats displayed a systematic shift in response from a choice of the larger delayed reward at short delays, to choice of the smaller immediate reward when the delay to the delivery of the larger reward was increased. A significant reduction in preference of the delayed reward was observed at the higher 20, 40 and 60 second delay conditions in comparison to choice trials when no delays were present. The delay discounting indicated is comparable to the pattern of choice observed previously in mice (Isles et al., 2003) pigeons (Mazur, 1987; Green et al., 2004) and humans (Rachlin et al., 1991; Green et al., 1994).

6.4.1. Stability of Task Performance

Results of Experiment 5A indicated that levels of impulsive choice remained stable across a three week period. These findings confirm and extend the findings of Evenden and Ryan (1996) and Cardinal et al. (2000) concerning long-term sensitivity to delayed reward in the paradigm. Moreover, the findings are consistent with more recent research in the human literature that has provided evidence of the stability of delay discounting over a three month period in a control student population (Ohmura et al., 2006). The speed of responding and frequency of omissions during task performance also displayed no evidence of significant variation during the 21 day period. The latency to initiate trials, make a reward choice and collect a reward were extremely rapid (all < 2 seconds), indicating that behavioural performance was under a high degree of stimulus control. In further support of this, omissions to initiate trials and to select and collect the rewards were minimal during performance of the task. The demonstration of stability of both choice behaviour and other task parameters in a control group of animals is crucial for later experiments of this thesis; this will enable any changes observed during chronic drug exposure, and initial and long-term drug withdrawal to be more confidentially attributed to the drug manipulations rather than changes in baseline performance.

A further important observation during the experimentation of this chapter was that, despite identical training on the task, extensive individual variability in impulsive choice was demonstrated at baseline (see Fig 6.6). The presence of individual differences in levels of impulsivity have been reported previously by authors adopting this particular delayed reward task (e.g. Cardinal et al., 2000; Winstanley et al., 2003a; Dellu-Hagedorn, 2006) and also other variant models, including adjusting amount, and delay procedures (Richards et al., 1997; Perry et al., 2005) and the T-maze delay discounting paradigm (Poulos et al., 1995). Based on the exponential model of decay, k estimates in animals in the present study ranged from 0.066 to 0.864, allowing a group of high (k range: 0.864 to 1.309) and low impulsive (k range: 0.066 to

0.280) animals to be identified. Supporting earlier research, the differences in learning ability were incapable of explaining the variation in rates of delay discounting, with both high and low impulsive animals requiring a comparable average of 41.5 ± 0.67 and 42.75 ± 2.31 training sessions respectively prior to the display of delay sensitive, stable choice behaviour in the task (Poulos et al., 1995; Deltu-Hagedorn, 2006). Taken together, these finding strongly suggest that the variability in performance at baseline may reflect differences in “trait” level of impulsiveness in rodents (Poulos et al., 1995; Perry et al., 2005).

These observed levels of “trait” discounting of delayed reward, as indicated by k , appear however to be considerably greater than that reported previously utilising a comparable model of delayed reward in rodents (k range: 0.011-0.140) (Winstanley et al., 2003). This suggests that the animals in the present research are substantially more impulsive. As both studies utilised Lister-hooded rodents, strain differences can not account for differences in the levels of discounting observed (e.g. Adriani et al., 2003). A difference across studies, however, was the level of food restriction adopted; animals in the study of Winstanley et al. were maintained on 14g of food per day in contrast to the 18.6g utilised in the present study. Previous research (Bradshaw and Szabadi, 1992; Wogar 1992; Ho et al., 1997), and data from the present study (see below), suggest that more severe, prolonged deprivation can lead to an increase in choice of delayed reward which may account for the reported lower levels of impulsive choice at baseline in previous research (Winstanley et al., 2003).

It is important to emphasise that the primary index of impulsivity in the paradigm is that of the percentage choice of delayed reward and the present research was not designed to compare competing models of delay discounting functions. Although the discounting of the delayed reward displayed by animals in the present research were well-described by the exponential model (see Table 6.2), it is highly plausible that other competing models such as the hyperbolic function may have additionally accounted for the data effectively (Richards et al., 1997; 1999a; Mazur, 2001). Comparison of decay functions that can account for choice behaviour within this model is an avenue for future research.

6.4.2. The Effects of Alterations in Primary Motivation on Task Performance

Nicotine is known to have effects on appetite (Grunberg, 1986; Grunberg et al., 1986; Miyata et al., 1999; Zhang et al., 2001). Given that later studies of this thesis assess the effects of acute and chronic nicotine administration and nicotine withdrawal on task performance, it was important to determine the effects of both increases and decreases in motivation for reward on performance in the delayed reward task. In agreement with previous research, acute prefeeding of normal rat chow and sucrose pellets had no significant effect on preference for delayed reward in the task (Logue and Pena-Correal, 1985; Logue et al., 1988; Richards et al., 1997;

Cardinal et al., 2000; Wade et al., 2000). Speed of responding parameters were however increased following prefeeding, indicating evidence of changes in motivation for reward. Consumption prior to testing of both normal rat chow and sucrose pellets, significantly increased the latency to initiate trials and respectively slowed the speed with which the immediate reward was chosen and the delayed reward was collected. These findings suggest that speed of responding measures and choice behaviour in the task can change independently. The lack of effect of a decrease in motivation on choice behaviour was also replicated following the prolonged increase in food intake. This was despite the 16 times greater increase in absolute BW across the seven day period relative to BL weight gain. In contrast to the acute feeding manipulations, longer term reduction in daily food intake was not associated with changes in the speed of responding in the task. These findings suggest that acute prefeeding displayed a greater magnitude of effect on speed of responding in comparison to prolonged reduction in deprivation level.

Conversely, acutely increasing the deprivation state of animals, significantly increased the overall choice of delayed reward in comparison to baseline choice behaviour. The enhanced preference of the delayed larger reward was delay-dependent, increasing choice significantly during the 10 second duration trials. The discounting of delayed reward was clearly less pronounced following the decrease in daily food intake, with only choice at the highest delay differing significantly from choice at the 0 second delay condition. Evidence of increased motivation for food reward was indicated by the significant reduction in the speed with which the delayed reward was collected and the reduction in failures to initiate trials. A comparable increase in the overall choice of delayed reward was observed following the prolonged increase in motivation for food reward, an effect that only reached significance during the final test session on the seventh day of the manipulation. The effect was not delay-dependent, although trends of an increase in preference of delayed reward in comparison to baseline were observed at the highest 40 and 60 second delay conditions. No other behavioural parameters in the task were affected by the increase in deprivation state, although the restricted daily feeding regime led to a significant (11.5g) loss in BW. Once again, in comparison to the acute-induced increase in motivation for reward, longer term increases in level of deprivation on task performance appear to be having less of an effect on performance. The increased preference for delayed reward observed following the acute and long-term increase in deprivation is in agreement with some (Bradshaw and Szabadi, 1992; Wogar 1992; Ho et al., 1997) but not all previous reports examining the effects of deprivation level on choice behaviour (Logue and Pena-Correal 1985; Logue et al., 1988; Cardinal et al., 2000; Wade et al., 2000). The variant delayed reward paradigms and contrasting food and water rewards utilised across research is likely to account for the discrepancy in findings.

Taken together, these findings have provided valuable comparator data of the effects of alterations in motivation for food reward which will facilitate interpretation of the effects of nicotine on performance of this task. The demonstration that choice behaviour was insensitive to decreases in motivation for the food reward, suggests that the anorectic effects of nicotine (Grunberg, 1986; Miyata et al., 1999; Zhang et al., 2001) are likely to play a minimal role in the drug-induced behavioural changes in the task. In contrast, the present findings suggest the need for caution when interpreting the effects of nicotine withdrawal on impulsive choice, during which, an associated increase in appetite and weight gain has been reported (Grunberg et al., 1986; Miyata et al., 1999). The present findings demonstrate that increasing motivation for the food reward leads to increases in the choice of the larger delayed reward and may be incorrectly inferred as being less impulsive. It is therefore crucial that the effects of increased motivation for food reward on task performance are considered during the interpretation of nicotine withdrawal on impulsive choice.

6.4.3. Delay Sensitivity

To determine the sensitivity of animals' choice behaviour to variations in the delay of reinforcement, a final manipulation involved the assessment of the effects of reducing the standard task delays on choice behaviour to 0, 1, 2, 5, and 15 seconds. This led to the substantial increase in overall choice of delayed reward (>80%). Under the delay manipulation, choice behaviour displayed minimal evidence of delay dependency, with choice of delayed reward failing to differ across delay conditions (see Fig.6.15). The substantial changes in choice behaviour confirm that performance in the delayed reward task is highly controlled by changes in delay. The fact that heightened sensitivity to delay was demonstrated even after a six month period of extensive training and testing indicates strong evidence that the pattern of choice behaviour within the task does not become a habitual response with longer term testing. Still evident during the delay manipulation however, was the characteristic within session shift in choice from the delayed to the immediate reward. This suggests that the passage of time, or progression of the session, may be a factor that, in addition to delay, may be affecting choice behaviour in the task. A limitation of the present research is that it does not effectively examine this theory. In order to explore this factor, complete omission of delays on choice behaviour could have been investigated or the effects of reversal of the normal sequence of delay conditions assessed. Research that has indeed investigated these manipulations in comparable paradigms reported that reversal of delay conditions leads to the alteration of choice of delayed reward in the opposite direction (Cardinal et al., 2000; Isles et al., 2003). The complete removal of delays profoundly increases the choice of delayed reward, although the shift from delayed to immediate reward remained, suggesting that to some extent the progression of the session may come to control rodent choice behaviour in the task with extensive testing (Evenden and Ryan, 1996; Cardinal et al., 2000).

6.4.4. Conclusions

In conclusion, the present studies demonstrated the long-term stability of choice behaviour and its sensitivity to delay, the lack of effect of decreases in primary motivation on impulsive choice, and the sensitivity of choice behaviour to increases in motivation for food reward. These data indicate the utility of the delayed reward task as an effective paradigm for the future investigation of effects of nicotine on impulsivity. Furthermore, the findings suggest caution must be given when interpreting the effects of nicotine withdrawal on task performance.

CHAPTER 7

Acute effects of nicotine on impulsive choice and the mediating role of the central nicotinic receptors

7.1. INTRODUCTION

7.1.1. General Introduction

Impulsive choice is considered a central mechanism in the acquisition, maintenance and relapse of drug addictive disorders. Drug dependent individuals frequently select the relatively brief but immediate reinforcing effects, or relief of withdrawal symptoms achieved from drug intoxication, over the longer term social and health benefits associated with a drug free life style (Madden et al., 1997).

Research using the DD task has shown that drug users discount the value of delayed rewards at a steeper rate than non-drug abusing controls. This has been reported for abusers of cocaine (Moeller et al., 2002; Coffey et al., 2003; Kirby and Petry, 2004), opioids (Madden et al., 1997; 1999; Kirby et al., 1999; Kirby and Petry, 2004) and alcohol (Vuchinich and Simpson, 1998; Petry, 2001). Demonstration has also been made of the pervasiveness of heightened impulsive choice in heavy smokers (Bickel et al., 1999; Mitchell, 1999; Reynolds et al., 2004; Reynolds, 2006a; Johnson et al., 2007). Greater sensitivity to delayed gratification in smokers in comparison to non-smokers has been demonstrated across differing magnitudes of reward, when considering both losses and gains (Baker et al., 2003), and when making choices regarding a range of commodities (Odum et al., 2005). Consistent with other drug dependent populations, smokers furthermore display more pronounced delay discounting of their drug of abuse (i.e. cigarettes) in comparison to other reward commodities. This finding suggests that the delayed drug of abuse loses its subjective value to a greater extent than other delayed rewards (Bickel et al., 1999; Baker et al., 2003; Johnson et al., 2007).

Whilst past research has left no doubt regarding the association between nicotine dependent individuals and impulsive choice, what remains unclear is to what extent the heightened sensitivity to delayed gratification leads to the initiation of smoking, or to what extent the heightened sensitivity to delayed gratification is a consequence of nicotine exposure. The demonstrated dose-dependent type relationship that has been shown between cigarette smoking and DD, suggests that the relationship is of a causal nature (Reynolds et al., 2004; Ohmura et al., 2005; Reynolds, 2006a; Johnson et al., 2007). One study that has attempted to explore the

direction of causality between impulsive choice and nicotine dependence by comparing adolescent smokers (smoked on average for 2.23 years), adult smokers (smoked on average for 5.00 years) and non-smokers (Reynolds, 2004). Reynolds argued that if impulsivity was a determining factor in the onset of nicotine addiction then both smoking groups should not differ in terms of impulsivity choice. Conversely, if long term cigarette consumption induces heightened impulsivity then adult smokers should display significantly greater rates of discounting in comparison to adolescent smokers. The results demonstrated that adult smokers discounted delayed monetary rewards to a greater degree than both non-smokers and young adolescent smokers. Adolescent smokers did not differ significantly from the control group, supporting the hypothesis that heightened impulsivity may be a consequence of long term nicotine exposure. For this interpretation to hold true however it must be assumed that all the adolescent smokers will continue smoking until adulthood.

In order to assess the theory that differences in impulsive choice between smokers and non-smokers arises due to the exposure of nicotine, research is necessary that explores directly the effects of the stimulant on DD. Despite the extensive research examining the effects of acute administration of stimulants such as cocaine and amphetamine (e.g. Cardinal et al., 2000; Paine et al., 2003; Helms et al., 2006), only one study has been conducted to assess the effects of nicotine on impulsive choice. Following the acute administration of nicotine (0.03-1.0mg/kg), Dallery and Locey (2005) reported a dose dependent increase in choice of the immediate smaller reward in a rodent adjusting delay paradigm. Caution, however, needs to be given when interpreting these findings, because the study consisted only of 5 subjects and the researchers failed to counterbalance the order of treatment doses across animals.

Although currently there is limited evidence to support the hypothesis that heightened impulsivity is a consequence of nicotine exposure, it is highly likely that nicotine impacts levels of impulsive choice due to the stimulants known ability via the nAChRs to alter the functioning of neurobiological systems believed to be involved in the mediation of the sensitivity to delayed reward (Di Chiara and Imperato, 1988; Nisell et al., 1996; Summer et al., 1996; Balfour et al., 1998; Mobini et al., 2000a; Cardinal et al., 2000; 2001; Adriani et al., 2004; Kheramin et al., 2004).

7.1.2. Objectives of Experiment 6 (A-C)

The primary objectives of Experiment 6 were therefore:

- i) To assess the acute effects of nicotine on impulsive choice as measured by the systematic delayed reward paradigm.
- ii) To determine whether the effects of acute nicotine on impulsive choice are mediated

through central nAChRs.

As in the experiments of Chapter 4, to achieve these objectives a dose response function for nicotine was initially determined on impulsive choice. As detailed previously (section 4.1.), nicotine acts via stimulating the nAChRs located throughout the CNS and peripheral nervous system. To establish whether nicotine's effects on impulsive choice were also mediated by central nicotinic receptors, the dose response function for the centrally acting nicotinic antagonist MEC was also examined. Finally, combination drug treatments of nicotine and 'silent' MEC were administered to assess whether MEC could antagonise the effects of nicotine in the delayed reward task.

7.2. EXPERIMENT 6 (A-C): EXAMINATION OF THE EFFECTS OF ACUTE ADMINISTRATION OF NICOTINE ON IMPULSIVE CHOICE AND CHARACTERISATION OF THE RECEPTORS MEDIATING THESE EFFECTS

7.2.1. METHOD

7.2.1.1. Subjects

Subjects were 11 adult male Lister Hooded rats (Charles River, UK). Animals were housed in pairs on arrival at the laboratory and maintained under a 12 hour light/dark cycle (lights on at 0700h) at controlled environmental temperature of $21^{\circ}\text{C} \pm 4^{\circ}\text{C}$ and relative humidity of $50\% \pm 10\%$. Across experiments food was restricted in order to maintain animals at 85% of their free feeding body weights. Water was available freely in home cages and feeding occurred at the end of each experimental day. At the start of testing, animals weighed approximately 350-380g. The same subjects were utilised for Experiments 6(A-C). Following the assessment of acute nicotine in Experiment 6A one subject failed to return to a stable baseline and was therefore removed from study. Only 10 subjects therefore completed Experiments 6B and 6C. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986.

7.2.1.2. Apparatus

Subjects conducted the task in two sets of four operant chambers (dimensions 30.5 X 24.1 X 21cm and 30.5 X 24.1 X 29.2cm, Med Associates Inc., USA). Refer to Chapter 2 section 2.5.1 for a detailed description of apparatus.

7.2.1.3. Behavioural Testing

The behavioural procedure of the delayed reinforcement task, in addition to the pre-training required has been described previously in the general methodology of Chapter 6, section 6.2.1.3. In this section also is the outline of behavioural measures assessed in the paradigm. Training continued on the task until subjects displayed choice behaviour that was delay sensitive and stable across a ten day period. Training was completed within approximately 10 weeks, during

which animals completed one session of the task per day.

7.2.1.4. Drugs

Both nicotine hydrogen tartrate salt and mecamylamine hydrochloride (MEC) were dissolved in 0.9% saline and administered s.c. in a volume of 1ml/kg body weight. All doses were calculated as free base and prepared freshly on each test day. The pH of all NIC drug solutions was adjusted to approximately 6, using 0.1M sodium hydroxide. Nicotine (0, 0.125, 0.25 and 0.5mg/kg) was administered s.c. 10 minutes prior to operant testing. The decision not to examine the effects of the higher 1.0mg/kg dose of nicotine was based upon the sedation and non-specific disruption of behaviour that was observed following its treatment in the go/no-go task (see Chapter 4, section 4.4). MEC (0, 0.1, 0.3 and 1.0mg/kg) was administered s.c. 20 minutes was prior to testing.

7.2.1.5. Design and Procedure

A within-subjects design was employed for experiments 6 (A-C). Treatment conditions were administered according to a Latin square design with a minimum of 72 hours separating consecutive drug treatments. It was ensured that animals had returned to BL performance prior to subsequent treatment doses being administered. Baseline was defined as impulsive choice deviating no more than 10% from the level of overall choice of delayed reward demonstrated by the subject prior to the initiation of Experiment 6A. Between experiments a one week 'wash out' period was given to minimise the possibility of drug carry over effects. During this period animals continued to perform the operant task once per day.

In the week prior to the initiation of drug testing, animals were habituated to injection procedures on two occasions, with subjects injected with 1ml/kg s.c. saline 10 minutes prior to the operant session. All experimentation followed standard operant testing procedures, outlined in detail in the general methodology of Chapter 2, section 2.5.2. All injection procedures were conducted in a procedure room separate from both the holding room and operant test laboratory. Testing took place during the light phase of their LD cycle between 0830h and 1400h. Experimentation was conducted over a 16 week period.

7.2.1.5.1. EXPERIMENT 6A: THE EFFECTS OF ACUTE ADMINISTRATION OF NICOTINE ON PERFORMANCE IN THE DELAYED REWARD TASK

The dose-response of nicotine on impulsive choice was first examined. Each of the four treatment conditions (0, 0.125, 0.25, and 0.5mg/kg s.c.) were administered 10 minutes prior to the test sessions following which they were transferred immediately to the operant test room. The sequence of nicotine doses was administered twice to each animal. Examination of each drug dose during two test sessions enabled a more accurate determination of the drug effects on

impulsive choice, through increasing the number of choice trials at each delay to twenty.

7.2.1.5.2. EXPERIMENT 6B: THE EFFECTS OF ACUTE ADMINISTRATION OF MECAMYLAMINE ON PERFORMANCE IN THE DELAYED REWARD TASK

Following Experiment 6A, a MEC dose response function (0, 0.1, 0.3 and 1.0mg/kg) was examined. As no difference in performance during time one and time two treatment of nicotine was observed on task performance each treatment of MEC was administered only once (see results section 7.3.3.2). The longer inter-injection interval for the antagonist meant that animals were first returned to their holding rooms until 10 minutes prior to testing, at which point they were transferred directly to the operant testing laboratory.

7.2.1.5.3. EXPERIMENT 6C: THE EFFECTS OF CO-ADMINISTRATION OF NICOTINE AND MECAMYLAMINE ON PERFORMANCE IN THE DELAYED REWARD TASK

Following Experiment 6B, nicotine and MEC were co-administered to determine whether the nicotinic antagonist was capable of blocking the effects of nicotine on the delayed reinforcement task observed in Experiment 6A. The 0.5mg/kg dose of nicotine was selected on the basis that this dose increased sensitivity to delay to the greatest extent across the nicotine dose range tested during Experiment 6A. Furthermore, this dose affected a range of behavioural parameters in the task, indicative of an increase in impulsive behaviour. MEC dose-response data from Experiment 6B demonstrated a lack of effect of the antagonist on impulsive choice across the dose range examined, enabling three 'silent' doses of the drug to be co-administered with nicotine. Treatment conditions during the co-administration experiment were saline/saline, saline/NIC_{0.5mg/kg}, MEC_{0.1mg/kg}/NIC_{0.5mg/kg}, MEC_{0.3mg/kg}/NIC_{0.5mg/kg} and MEC_{1.0mg/kg}/NIC_{0.5mg/kg}.

Saline/saline and saline/ NIC_{0.5mg/kg} drug combinations served as treatment controls. Saline/saline, controlled for the effects of combination injections on behaviour, whilst the combined administration of saline/ NIC_{0.5mg/kg}, acted as a positive drug control. The first of all combination treatments was administered 20 minutes prior to testing, following which animals were returned to their holding room. Ten minutes prior to the testing session, animals were then returned to the preparation room where the second treatment was administered after which they were transferred directly to the operant testing room. All drug combinations were administered once.

7.2.1.6. Statistical Analysis

To assess the sensitivity to delay and stability of baseline behaviour prior to drug testing, data from ten consecutive test sessions was analysed using a two-way repeated measures ANOVA, with both day and delay as the within subject factors. A significant main effect of delay would indicate sensitivity of choice behaviour across delays, while a non-significant main effect of day

would demonstrate evidence of stability of this choice.

For all drug experiments behavioural parameters, including overall choice of delayed reward (CDR), speed of responding and omissions were analysed using a one-way repeated measures ANOVA. Analysis of the choice of reward by delay, was conducted using a two-way repeated measures ANOVA, with both treatment condition and delay (0, 10, 20, 40 and 60s) as the within subject variables. During experiment 6A, each dose of nicotine was administered twice; data therefore from both test sessions was averaged for each treatment dose prior to analysis.

Due to the repeated administration of nicotine during experiment 6A, dose response data on choice behaviour was compared from time one administration to time two. This analysis was conducted to assess whether prior nicotine exposure altered subsequent response to nicotine. A three-way ANOVA was applied to the data with treatment, delay and time as the within subject factors.

Choice behaviour on the task during non-drug baseline days was compared across Experiments 6A-C. This analysis was conducted to determine the stability of BL impulsive choice across the 16 weeks of testing. Independently for each experiment, choice behaviour across delays during the BL test session immediately prior to each treatment dose was averaged. The average baseline for Experiment 6A, 6B and 6C was then analysed by a two-way repeated measures ANOVA with Experiment and delay as the within subject factors.

All significant main effects of analysis were assessed further by Bonferroni post hoc comparisons. Significant interactions were examined further by simple effects analysis. In the case of significant treatment x delay interactions, simple one-way ANOVAs were used to examine both the main effects of treatment dose across each of the five delay conditions and the main effects of delay at individual treatment doses. Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction. All data prior to analysis was assessed for normality and transformed where necessary (see also section 2.7). If data could not be successfully transformed, then the non-parametric Friedman test was employed, followed where appropriate by Wilcoxon Signed Ranks tests. In all cases of analysis α values of $p < 0.05$ were deemed statistically significant.

7.3. RESULTS

Further supplementary statistical information (ANOVA tables) can be found in Appendix 2. Across experiments 6(A-C) omissions of both lever selection and collection of reward following an immediate choice were not analysed due to both behaviours rarely occurring.

7.3.1. Baseline Performance: Pre-drug Testing

Prior to the initiation of drug testing analysis of choice behaviour across ten consecutive sessions demonstrated that behaviour was both stable ($F(9,90) = 0.337$, N.S) and delay sensitive across animals ($F(4,40) = 52.301$, $p < 0.001$). Post hoc analysis revealed that the greatest sensitivity to delay was during 40 and 60 second delays. During these trials choice for the immediate reward was significantly greater in comparison to when no delays were present during choice (all $p < 0.01$). No significant delay x day interaction was found ($F(36, 360) = 1.429$, N.S).

7.3.2. Baseline Performance: During Drug Testing

Baseline choice behaviour across delay remained consistent across Experiments with no main effect of Experiment ($F(2,18) = 0.519$, N.S) or experiment x delay interaction observed ($F(8,72) = 0.409$, N.S). Baseline choice behaviour furthermore remained highly sensitive to delay ($F(2.251, 20.259) = 66.041$, $p < 0.001$), with significant reductions in choice of the larger delayed reward remaining at 40 and 60 seconds delay in comparison to when no delays were present (all $p < 0.01$) (see Fig.7.1).

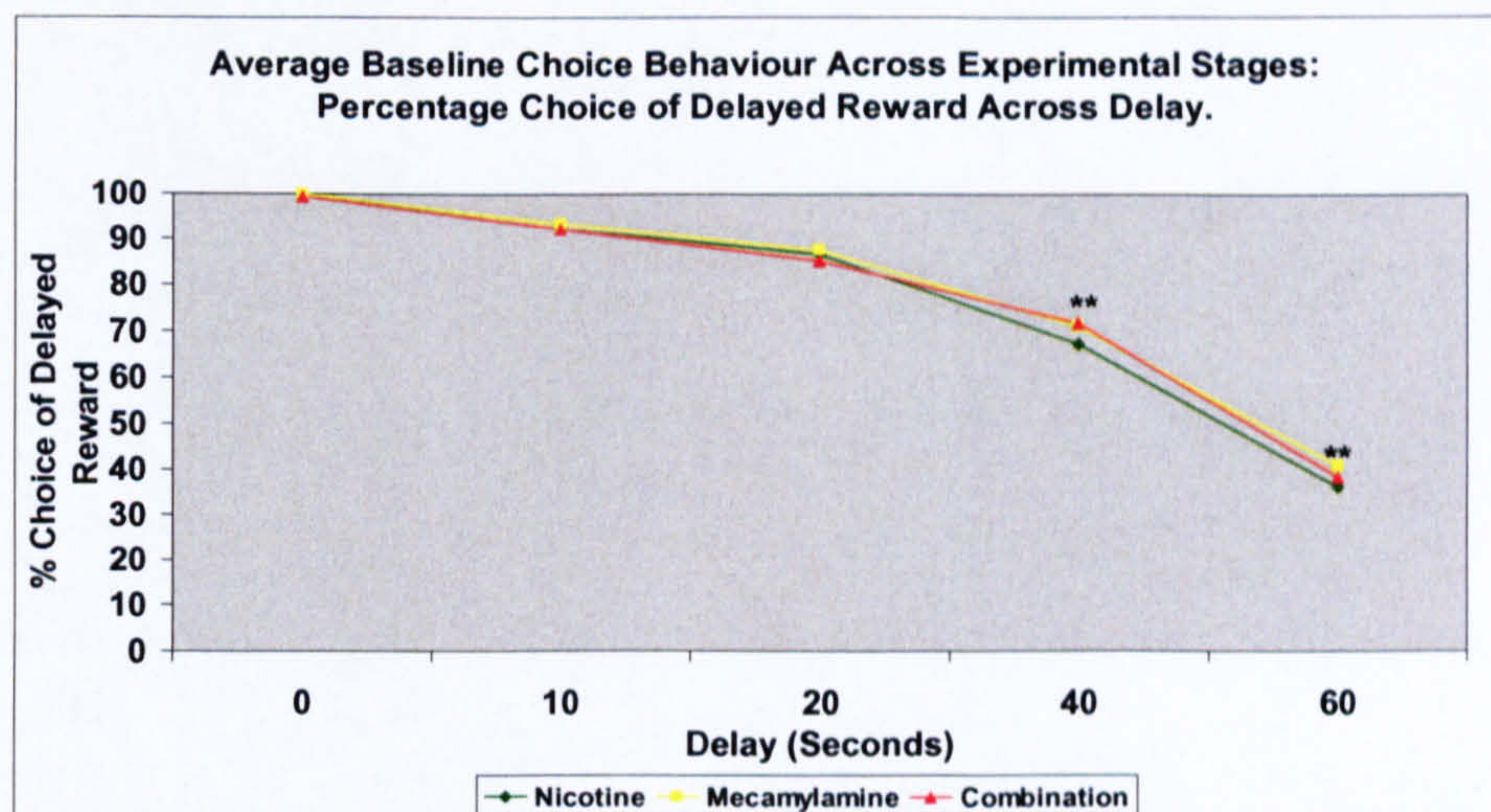


Fig. 7.1: Comparison of baseline choice behaviour across delay of Experiments 6 A-C

7.3.3. EXPERIMENT 6A: THE EFFECTS OF ACUTE ADMINISTRATION OF NICOTINE ON PERFORMANCE IN THE DELAYED REWARD TASK

7.3.3.1. Choice Behaviour

A marked dose-dependent decrease in the choice of the delayed reward was found following the acute administration of nicotine ($F(3,30) = 30.478$, $p < 0.001$). As illustrated in Fig. 7.2, all doses administered significantly decreased overall choice of delayed reward in comparison to the saline control, with the greatest reduction in choice of delayed reward being observed at the

highest 0.5mg/kg dose (all $p < 0.01$). Furthermore, the choice of delayed reward at the 0.5mg/kg dose was significantly lower than the 0.125mg/kg and 0.25mg/kg treatment doses (all $p < 0.01$).

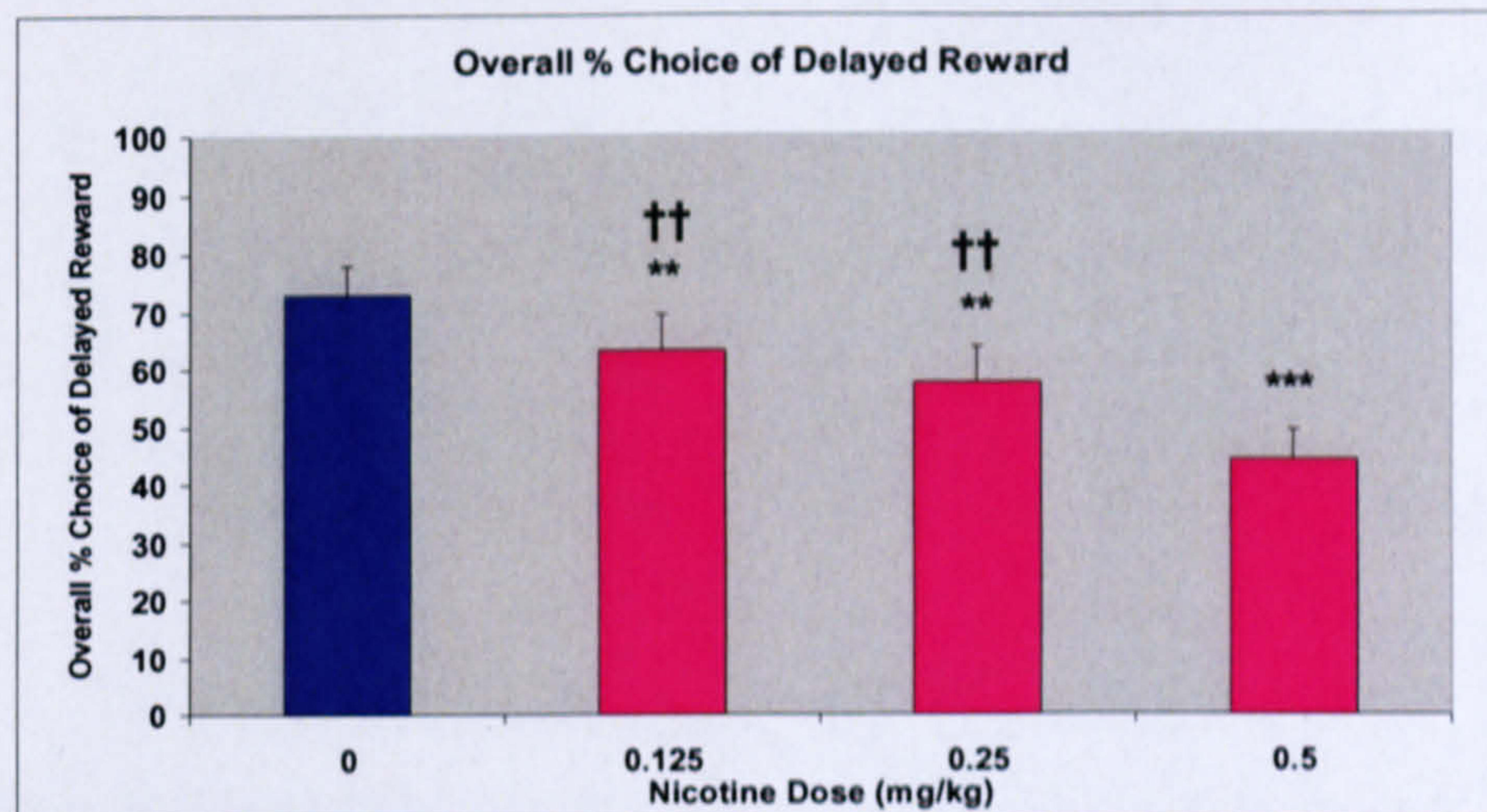


Fig. 7.2: The effects of acute nicotine on overall percentage choice of delayed reward. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

Analysis of choice by delay once again demonstrated a highly significant main effect of treatment dose ($F(3,30) = 25.610$, $p < 0.001$). A main effect of delay was furthermore found ($F(1.653, 16.528) = 74.188$, $p < 0.001$), indicating that preference for the delayed larger reward was significantly lower at the 20, 40 and 60 second delay conditions in comparison to the 0 second delay condition (all $p < 0.01$). Analysis revealed a significant treatment dose \times delay interaction, suggesting that treatment doses differentially affected choice of the larger reward by delay ($F(12,120) = 5.016$, $p < 0.001$). To examine the interaction further a series one-way ANOVAs assessing the main effect of treatment at each delay were conducted (see Fig. 7.3). No significant main effect of treatment was observed at the 0 second delay condition with the predominate choice being that of the larger reward across drug treatments at this delay ($F(3,30) = 0.897$, N.S.). Conversely, significant main effects of nicotine treatment at the 10, 20, 40 and 60 second delay conditions were found (all $F(3,30) \geq 6.550$, $p \leq 0.002$). Post hoc comparisons revealed that following the lowest 0.125mg/kg dose, choice of delayed reward was significantly lower only during 40 second delay trials in comparison to choice behaviour at this delay following saline control ($p < 0.05$). Following treatment of the 0.25mg/kg dose, choice of the delayed reward in comparison to saline control was significantly lower at both the 40 and 60 second delay conditions (all $p < 0.01$). At the highest 0.5mg/kg dose choice was decreased at 10, 20, 40 and 60 second delay conditions in comparison to choice at these delays following saline (all $p < 0.01$). Furthermore, the reduction in choice at 10 seconds following 0.5mg/kg dose reached significance in comparison to the lowest 0.125mg/kg dose ($p < 0.05$). Decreased choice during the 20 second delay trials additionally reached significance in comparison to the

0.125mg/kg and 0.25mg/kg doses (all $p < 0.05$).

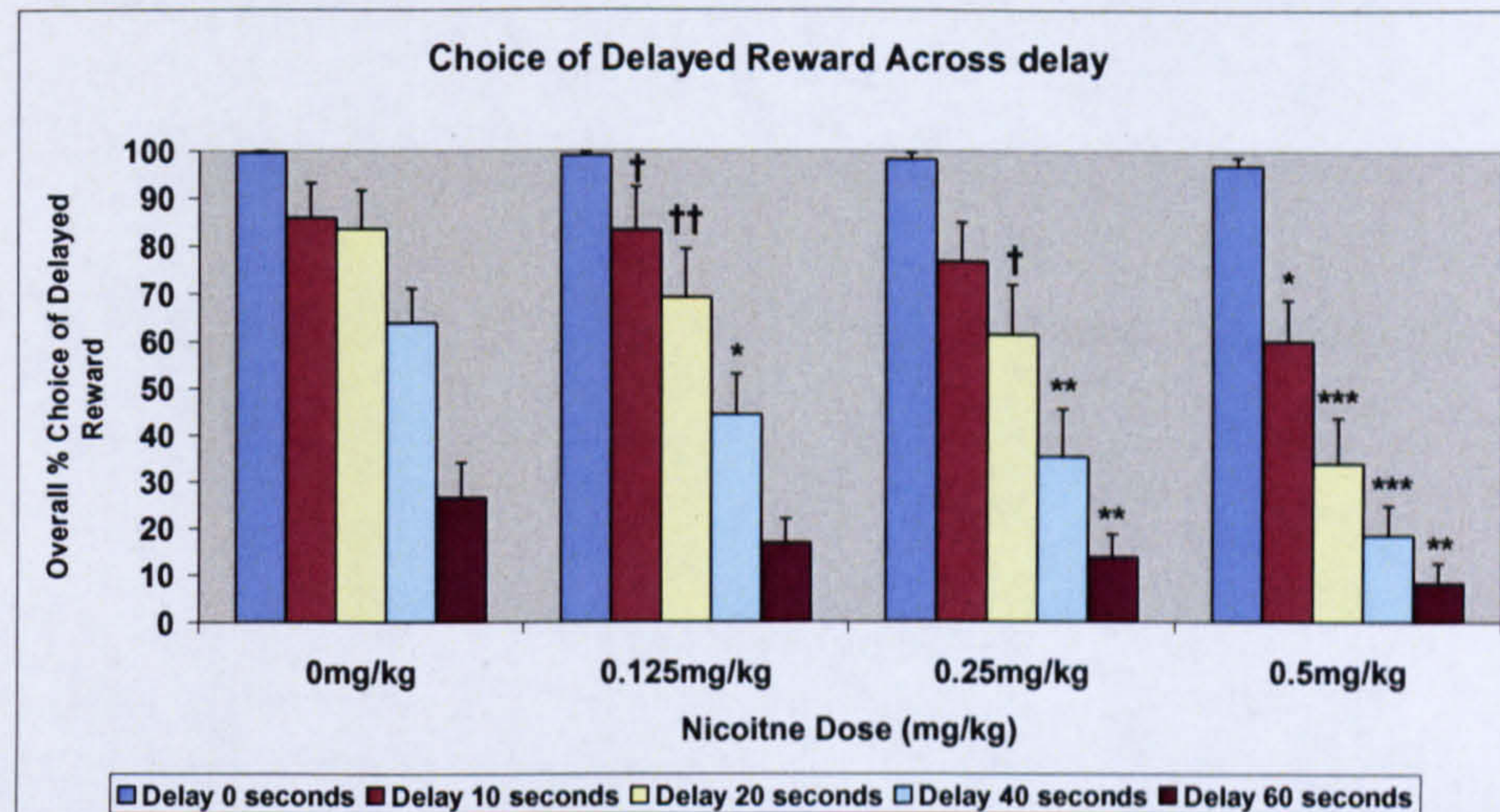


Fig. 7.3: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on percentage choice of delayed reward across delay condition. Treatment effects across each delay. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5mg/kg nicotine dose.

The significant dose \times delay interaction was further analysed by examining the main effect of delay at individual test doses. As illustrated in Fig. 7.4, all doses displayed a significant main effect of delay (all $F(4,36) \geq 34.477$, $p \leq 0.001$), with a dose related reduction in choice of delayed reward across 10, 20, 40 and 60 second delay conditions following nicotine treatment. Analysis of choice behaviour across delay during saline treatment displayed a significant reduction of choice of delayed reward at 40 and 60 seconds delay in comparison to when there was no delay following the selection of the larger reward (all $p < 0.01$). A similar behavioural pattern of choice of delayed reward was shown at the lowest 0.125mg/kg treatment of nicotine where the greatest intolerance to delay was also seen at 40 and 60 second delay conditions (all $p < 0.01$). Following 0.25mg/kg choice of delayed reward was significantly lower at 20, 40 and 60 delay conditions in comparison to the 0 second delay condition (all $p < 0.05$). Under the influence of the highest 0.5mg/kg nicotine dose, post hoc analysis indicated that choice of the delayed reward was significantly lower across all delays in comparison to the 0 second delay condition (all $p < 0.01$).

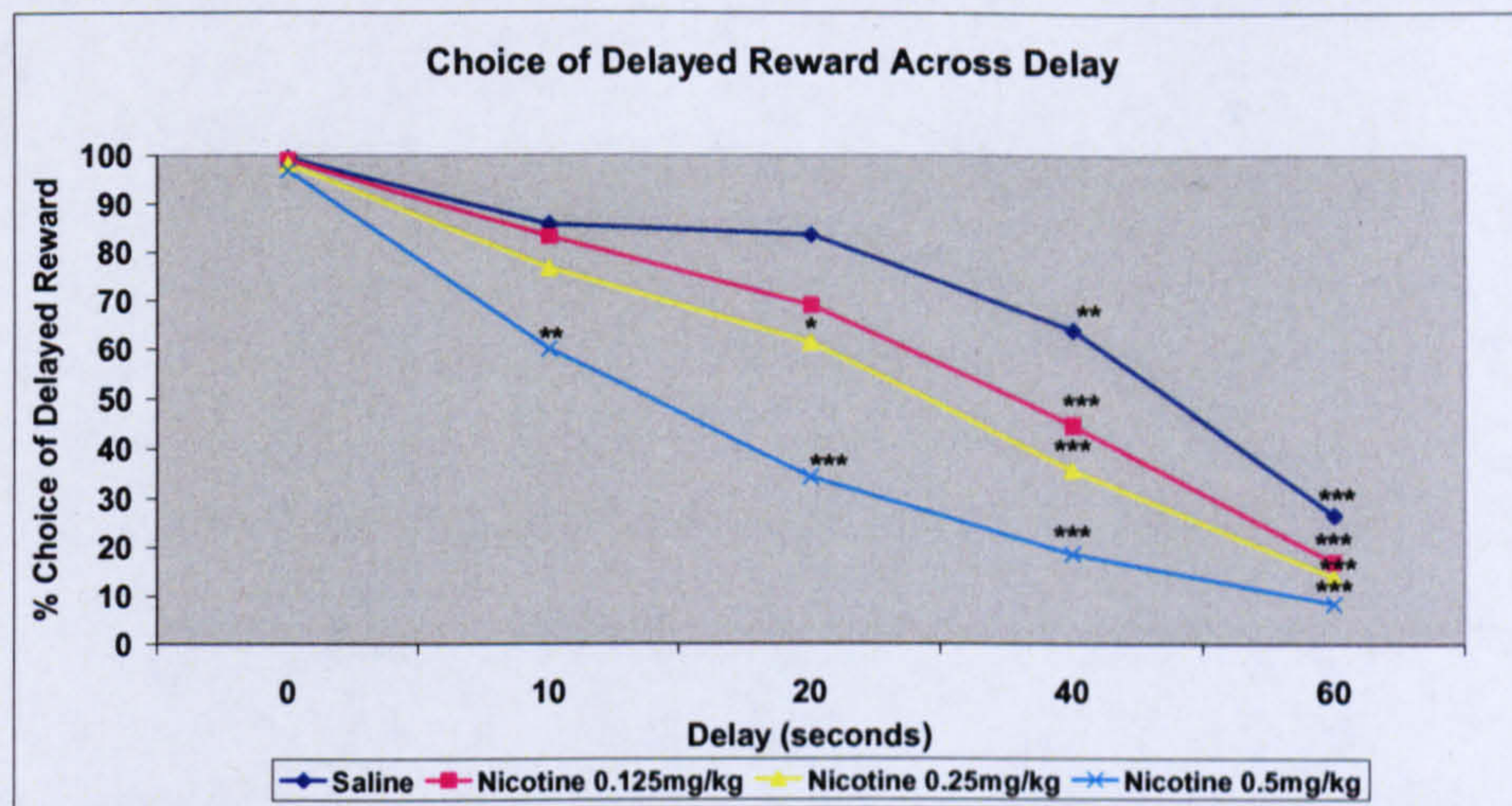


Fig. 7.4: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. Each point represents the mean. *, $p < 0.05$. **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

7.3.3.2. Comparison of Administration of Nicotine at Time One and Time Two

No differences in response to nicotine were observed on choice of delayed reward across delay at time one and time two administration of treatment. This was supported by the non significant main effect of time ($F(1,10) = 0.187$, N.S.) and the lack of significant time x treatment ($F(3,30) = 1.129$, N.S.), time x delay ($F(4,40) = 0.845$, N.S.) and time x treatment x delay ($F(12,120) = 0.624$, N.S.) interactions.

7.3.3.3. Speed of Responding

Nicotine treatment was found to decrease the latency to initiate trials in the task ($F(3,30) = 8.101$, $p < 0.001$). This effect reached significance at the 0.25mg/kg ($p < 0.05$) and 0.5mg/kg ($p < 0.01$) doses of nicotine in comparison to the control treatment condition (see Fig. 7.5). The speed with which animals made a choice between rewards, however, was not affected following nicotine treatment. This lack of effect was evident for both the latency to choose the immediate ($F(3,30) = 1.631$, N.S.) and delayed reward ($F(1.535, 15.351) = 1.875$, N.S.).

The speed with which animals collected food reward following an immediate choice was not affected by the acute administration of nicotine ($F(3,30) = 0.118$, N.S.). In contrast, following a delayed reward choice 0.5mg/kg nicotine significantly reduced the latency to collect the reward ($X^2 = 11.182$, $df = 3$, $p = 0.011$). As well as differing from control ($p < 0.01$), the 0.5mg/kg dose in addition reduced significantly delayed magazine latency relative to 0.25mg/kg nicotine dose ($p < 0.05$). Table 7.1 summarises the effects of the acute administration of nicotine on speed of responding in the delayed reward task.

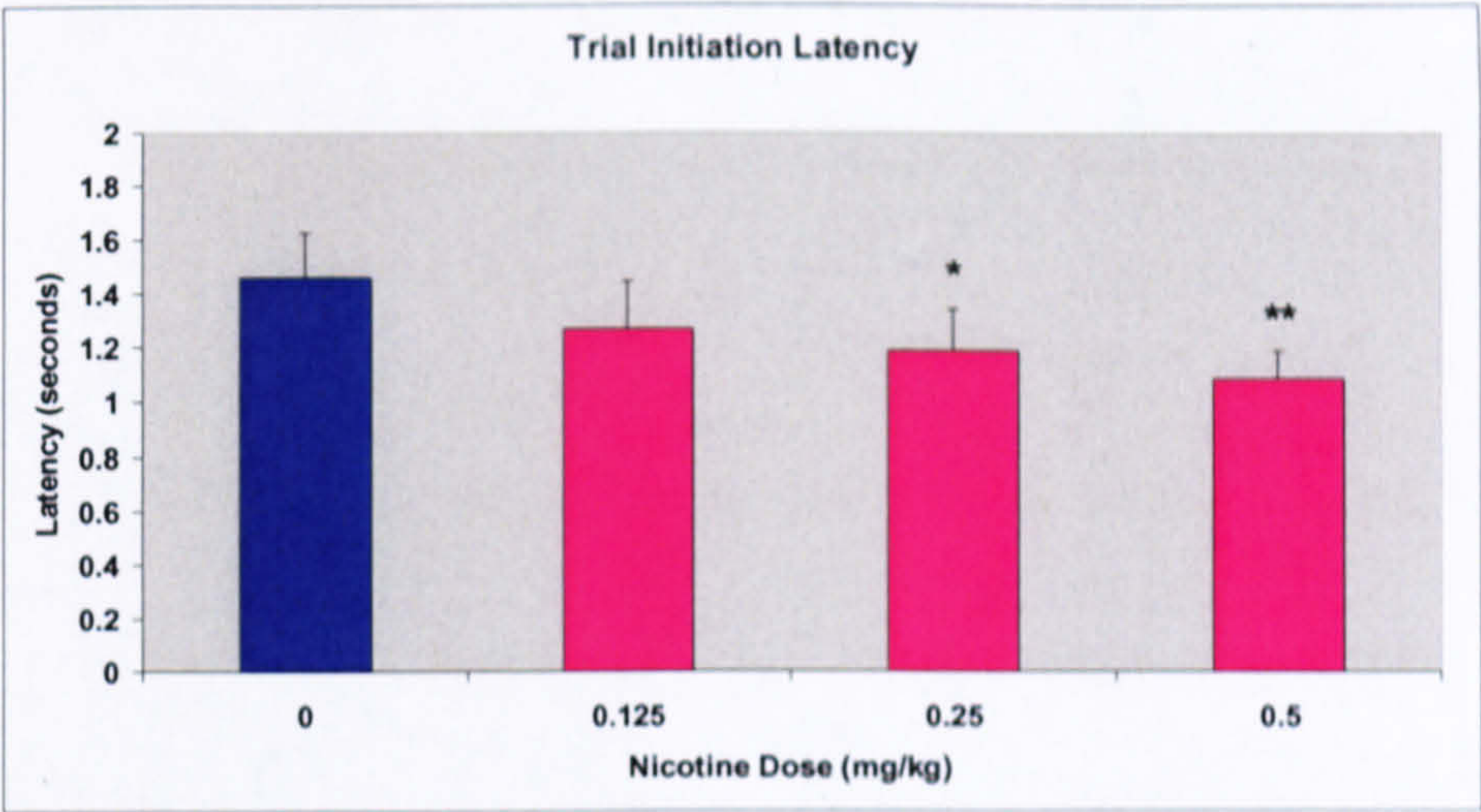


Fig. 7.5: The effects of acute nicotine on trial initiation latency. Each bar represents the mean latency ± SEM. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ (Bonferroni comparison) as compared to saline control.

7.3.3.4. Omissions

No significant effect of treatment was observed on omissions to initiate trials ($X^2 = 5.947$, $df = 3$, N.S). At the highest 0.5mg/kg dose however a significant decrease in magazine omissions to collect delayed reward was shown ($X^2 = 9.720$, $df = 3$, $p=0.021$). Table 7.2 summarises the effects of nicotine treatment on omissions.

Table 7.1: The effect of acute nicotine on speed of responding in the delayed reward task

Behavioural Measure	Nicotine Dose (mg/kg)			
	Saline (control)	0.125	0.25	0.5
Immediate Response Latency	0.61 ± 0.03	0.63 ± 0.03	0.62 ± 0.02	0.66 ± 0.03
Delayed Response Latency	0.73 ± 0.03	0.67 ± 0.03	0.73 ± 0.04	0.78 ± 0.06
Immediate Magazine Latency	0.29 ± 0.05	0.27 ± 0.04	0.26 ± 0.03	0.27 ± 0.04
Delayed Magazine Latency	1.09 ± 0.21	0.93 ± 0.20	0.90 ± 0.29†	0.64 ± 0.20**

Table 7.1: Each value represents the mean latency (seconds) ± SEM. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ (Bonferroni comparison) as compared to saline control. †, $p<0.05$, ††, $p<0.01$, †††, $p<0.001$ (Bonferoni comparison) as compared to highest 0.5mg/kg nicotine dose.

Table 7.2: The effect of acute nicotine on omissions in the delayed reward task

Behavioural Measure	Nicotine Dose (mg/kg)			
	Saline (control)	0.125	0.25	0.5
Trial Omissions	0.27 ± 0.18	0.32 ± 0.15	0.14 ± 0.10	0.59 ± 0.24
Magazine Omissions Following Delayed Choice	1.23 ± 0.49	1.09 ± 0.48	0.73 ± 0.50	0.45 ± 0.31*

Table 7.2: Each value represents the mean frequency ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 (Bonferroni comparison) as compared to saline control.

7.3.4. EXPERIMENT 6B: THE EFFECTS OF ACUTE ADMINISTRATION OF MECAMYLAMINE ON PERFORMANCE IN THE DELAYED REWARD TASK

7.3.4.1. Choice Behaviour

A significant main effect of treatment was observed on overall choice of delayed lever following treatment with MEC ($F(3,27) = 30.478, p=0.011$). Post hoc analysis however revealed that no drug treatments differed significantly from control. The main effect instead reflected evidence of a marginal, though significant, difference between the highest, 1.0mg/kg dose, where an decrease in choice of delayed reward was shown, in comparison to 0.1mg/kg treatment ($p<0.05$) (see Fig 7.6).

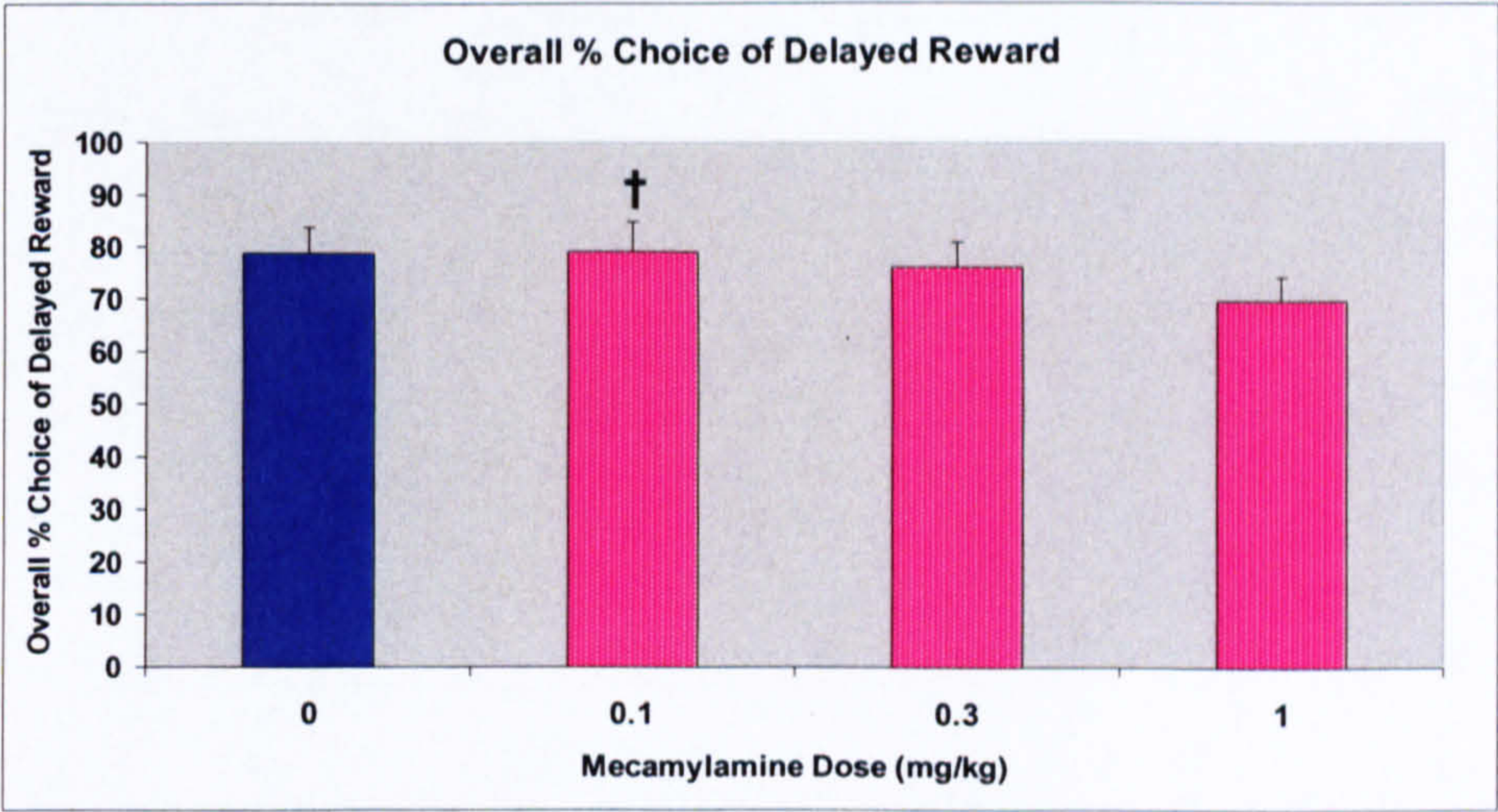


Fig. 7.6: The effects of acute mecamylamine on overall percentage choice of delayed reward. Each bar represents the mean percentage ± SEM. †, p<0.05, ††, p<0.01, †††, p<0.001 (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

Analysis of choice by delay again demonstrated a main effect of treatment ($F(3,27) = 3.246, p=0.037$). Post hoc comparisons however displayed no significant differences between drug treatments. A highly significant main effect of delay was additionally revealed ($F(2.05, 18.493)$

= 31.101, $p < 0.001$), indicating that choice of the delayed reward was significantly lower at 40 and 60 second delay conditions in comparison to the 0 delay condition (all $p < 0.05$). A significant dose x delay interaction was also found ($F(12, 108) = 2.098$, $p = 0.028$). As shown in Fig. 7.7, further analysis of the interaction examining the main effect of treatment at each delay, indicated that choice of delayed reward did not differ following treatment at 0, 10, 40 and 60 second delays (all $F(3,27) \leq 2.665$, N.S). A significant main effect of treatment however was shown at the 20 second delay ($F(3,27) = 4.063$, $p = 0.017$). Post hoc analysis at this delay revealed that choice behaviour following treatment did not differ from saline, but instead the highest dose of MEC decreased preference for the delayed reward in comparison to the 0.3mg/kg treatment dose ($p = 0.05$).

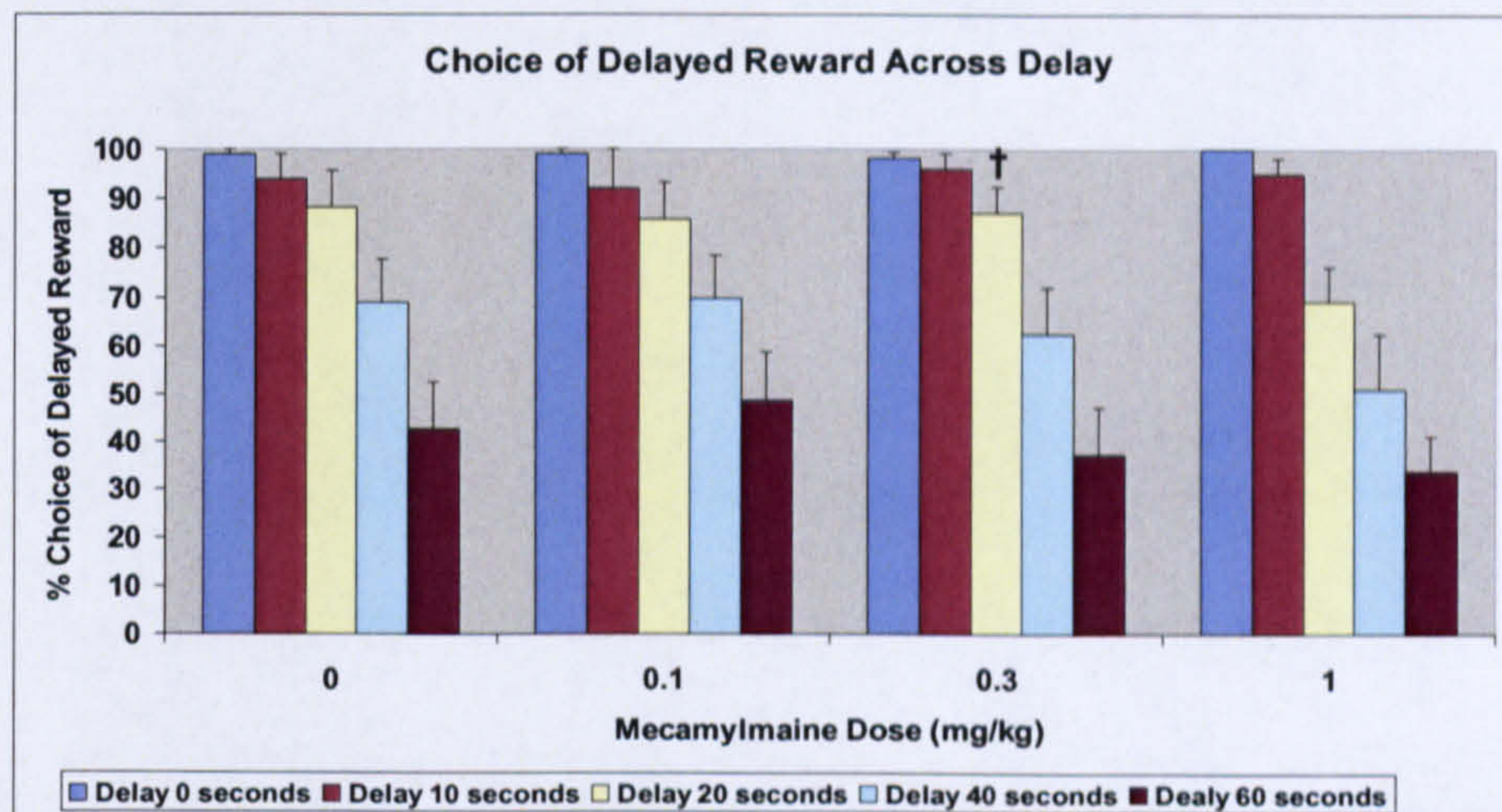


Fig. 7.7: Simple effects analysis of treatment*delay interaction: The effects of acute mecamylamine on percentage choice of delayed reward across delay condition. Treatment effects across each delay. Each bar represents the mean percentage \pm SEM. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 1.0mg/kg nicotine dose.

Exploration of interaction through assessment of the main effect of delay across individual test doses revealed that choice across all treatments of the antagonist were sensitive to delay (all $F(4,36) \geq 16.24$, $p \leq 0.001$). Saline treatment, 0.1 and 0.3mg/kg MEC significantly decreased choice of the delayed reward at 40 and 60 seconds in comparison to the zero delay condition (all $p < 0.05$) (see Fig.7.8). Choice of the delayed reward following 1.0mg/kg MEC significantly decreased choice at 20, 40 and 60 seconds delay in comparison to the 0 second delay condition (all $p < 0.05$) (see Fig.7.8).

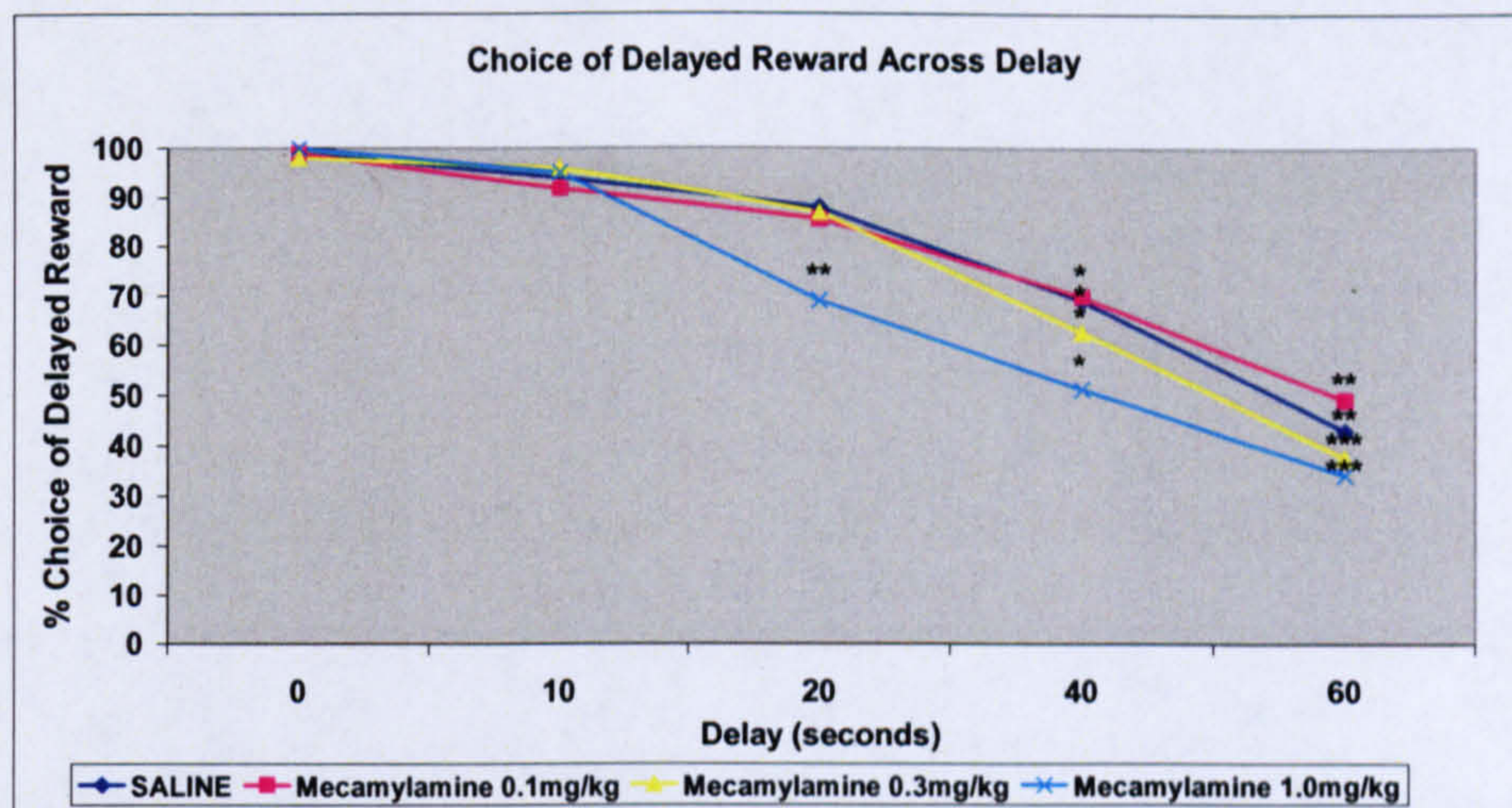


Fig. 7.8: Simple effects analysis of treatment*delay interaction: The effects of acute mecamylamine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

7.3.4.2. Speed of Responding

Table 7.3 summarises the effect of the nicotinic antagonist on speed of responding in the task. MEC did not affect the latency to initiate trials ($F(3,26) = 0.487$, N.S.), the latency with which animals chose either the delayed or immediate reward (all $F(3,27) \leq 1.381$, N.S.) or the speed with which animals collected reward following either an immediate or delayed reward choice ($F(1.722, 15.497) = 1.381$, N.S; $F(3,27) = 0.286$, N.S., respectively).

7.3.4.3. Omissions

Administration of MEC did not affect the frequency of trial omissions or failure to collect reward following a delayed reward choice (all $X^2 \leq 1.737$, $df = 9$, N.S) (see table 7.4).

Table 7.3: The effect of acute mecamylamine on speed of responding in the delayed reinforcement task

Behavioural Measure	Mecamylamine Dose (mg/kg)			
	Saline (control)	0.1	0.3	1.0
Trial Initiation Latency	1.43 ± 0.14	1.66 ± 0.20	1.53 ± 0.15	1.55 ± 0.15
Immediate Response Latency	0.68 ± 0.06	0.71 ± 0.04	0.66 ± 0.04	0.68 ± 0.04
Delayed Response Latency	0.73 ± 0.03	0.75 ± 0.05	0.72 ± 0.04	0.72 ± 0.03
Immediate Magazine Latency	0.30 ± 0.08	0.28 ± 0.05	0.29 ± 0.05	0.31 ± 0.07
Delayed Magazine Latency	0.92 ± 0.20	0.94 ± 0.22	1.15 ± 0.33	0.96 ± 0.23

Table 7.3: Each value represents the mean latency ± SEM.

Table 7.4: The effect of acute mecamylamine on omissions in the delayed reinforcement task

Behavioural Measure	Mecamylamine Dose (mg/kg)			
	Saline (control)	0.1	0.3	1.0
Trial Omissions	0.80 ± 0.33	1.10 ± 0.60	0.90 ± 0.41	0.70 ± 0.30
Magazine Omissions Following Delayed Choice	1.30 ± 0.62	1.50 ± 0.70	2.00 ± 1.02	0.90 ± 0.41

Table 7.4: Each value represents the mean frequency ± SEM.

7.3.5. EXPERIMENT 6C: THE EFFECTS OF CO-ADMINISTRATION OF NICOTINE AND MECAMYLAMINE ON PERFORMANCE IN THE DELAYED REWARD TASK

7.3.5.1. Choice Behaviour

Analysis of overall choice of delayed reward demonstrated a significant main effect of treatment ($F(4,26) = 17.487$, $p < 0.001$) (see Fig.7.9). Nicotine treatment alone significantly reduced choice of the delayed reward in comparison to saline control ($p < 0.01$). Co-administration of nicotine with 0.1mg/kg MEC, furthermore, significantly reduced overall choice of the delayed reward in comparison to control treatment ($p < 0.05$). Following co-administration of nicotine and 0.3mg/kg MEC, however, the choice of the delayed reward did not differ from saline treatment and was furthermore significantly greater than choice of delayed reward following

nicotine treatment alone ($p < 0.01$). Finally, the co-administration of nicotine and 1.0mg/kg MEC dose significantly decreased choice of the delayed reward in comparison to saline treatment. Following the co-administration of nicotine with both 0.1 and 1.0mg/kg MEC, overall choice of delayed reward was furthermore significantly lower than choice of the delayed reward following pre-treatment with 0.3mg/kg MEC (all $p < 0.05$) (see Fig. 7.9).

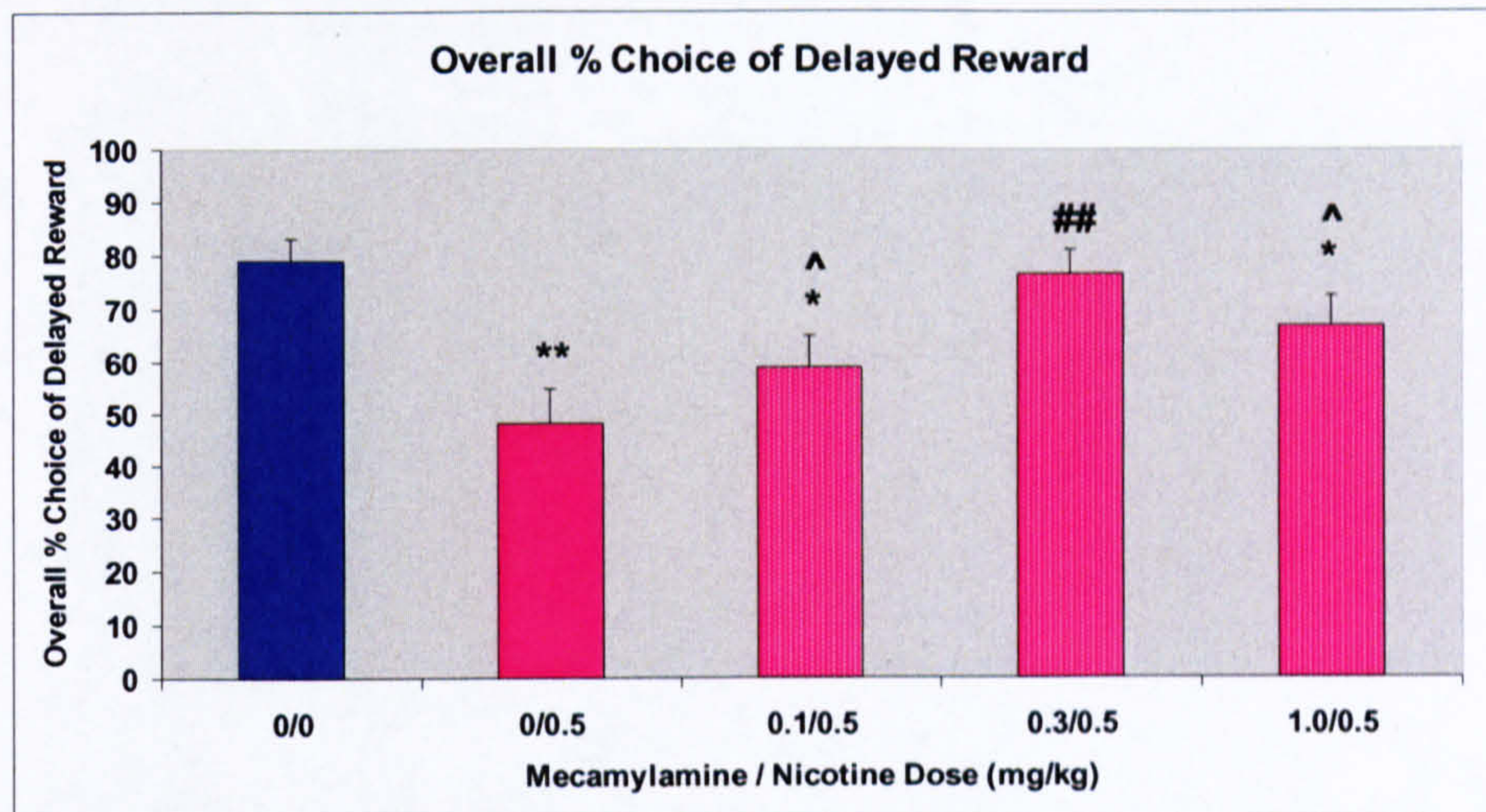


Fig. 7.9: The effects of co-administration of nicotine and mecamylamine on overall percentage choice of delayed reward. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control (0/0mg/kg dose). #, $p < 0.05$, ##, $p < 0.01$, ###, $p < 0.001$ (Bonferroni comparison) as compared to nicotine treatment alone (0/0.5mg/kg dose). ^, $p < 0.05$, ^^, $p < 0.01$, ^^^, $p < 0.001$ (Bonferroni comparison) as compared to combined treatment of 0.3mg/kg mecamylamine and nicotine (0.3/0.5mg/kg dose).

The effects observed following co-administration of nicotine and MEC are further supported by the significant main effect of treatment following analysis of choice by delay ($F(4,36) = 20.248$, $p < 0.001$). A significant main effect of delay was also observed ($F(2,315, 20.839) = 59.861$, $p < 0.001$), indicating that choice of delayed reward was significantly reduced during the 20, 40 and 60 second delay trials in comparison to the 0 second delay condition (all $p < 0.05$). Analysis additionally revealed a dose \times delay interaction ($F(12,120) = 2.317$, $p = 0.005$) (see Fig. 7.10). Further assessment revealed that the main effects of treatment across delay conditions reached significance at all delays with exception of the 0 second delay condition (all $F(4,36) \geq 4.742$, $p \leq 0.004$). Post hoc analyses revealed that nicotine treatment alone significantly reduced choice of the delayed larger reward at 20 and 40 second delays in comparison to saline (all $p < 0.05$). In contrast, examination of pre-treatment with 0.1 and 1.0mg/kg MEC decreased choice of the delayed reward significantly at the 20 seconds delay only in comparison to saline control (all $p < 0.05$). However, choice of reward across all delays following the co-administration of nicotine and both 0.1mg/kg and 1.0mg/kg did not differ from that of choice following the administration of nicotine alone. In contrast, following co-administration of nicotine and 0.3mg/kg MEC, choice of delayed reward across all delays did not differ from saline control (all

$p > 0.05$). Choice of delayed reward, however, at 20, 40 and 60 seconds delays was significantly greater than choice at these delays following nicotine treatment alone. Pairwise comparisons also highlighted that following pre-treatment with 0.3mg/kg MEC, choice of delayed reward during the 10 seconds delay condition was significantly greater than choice at this delay following pre-treatment with the lowest 0.1mg/kg MEC dose.

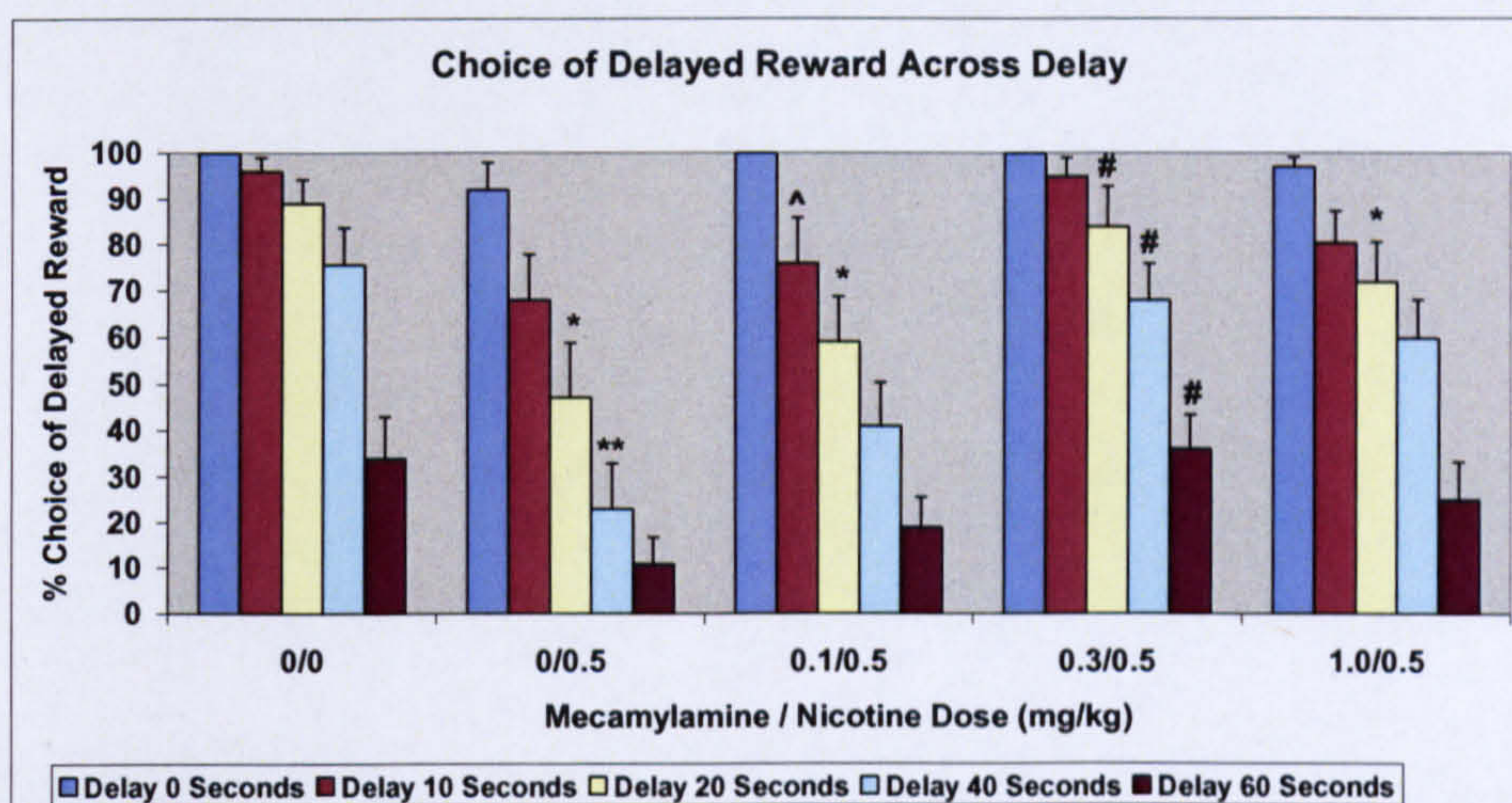


Fig. 7.10: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on percentage choice of delayed reward across delay condition. Treatment effects across each delay (a) and rate of discounting at individual doses (b). Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control (0/0mg/kg dose). #, $p < 0.05$, ##, $p < 0.01$, ###, $p < 0.001$ (Bonferroni comparison) as compared to nicotine treatment alone (0/0.5mg/kg dose). ^, $p < 0.05$, ^^, $p < 0.01$, ^^^, $p < 0.001$ (Bonferroni comparison) as compared to combined treatment of 0.3mg/kg mecamylamine and nicotine (0.3/0.5mg/kg dose).

Analysis of the dose \times delay interaction was further explored through a series of one-way ANOVAs assessing the main effects of delay across single combination doses. All treatment conditions resulted in choice behaviour that was sensitive to increasing delays (all $F(4,36) \geq 23.101$, $p \leq 0.001$) (see Fig. 7.11). Post hoc analysis of choice behaviour under saline treatment revealed that choice of delayed reward was significantly reduced at 40 and 60 second delays in comparison to choice behaviour during the 0 delay condition (all $p < 0.05$). When, nicotine was administered alone, choice of the delayed reward was significantly lower across all delays in comparison to choice of reward when no delays were present (all $p < 0.05$). Following pre-treatment with 0.1mg/kg MEC, choice behaviour was similar to that of nicotine treatment alone, with choice of the delayed reward being significantly reduced at 20, 40 and 60 second delay conditions in comparison to choice at the 0 delay (all $p < 0.01$). However, following the co-administration of nicotine and 0.3mg/kg MEC choice across delays was most similar to saline treatment, with choice of delayed reward only reaching significance at 40 and 60 seconds in comparison to the 0 delay condition (all $p < 0.01$) (see Fig. 7.11). Intermediate between saline control and nicotine treatment alone was the choice across delays following pre-treatment with the highest 1.0mg/kg MEC. Choice of the delayed reward was decreased at 40 and 60 second delay conditions in comparison to choice at the zero delay condition (all $p < 0.01$).

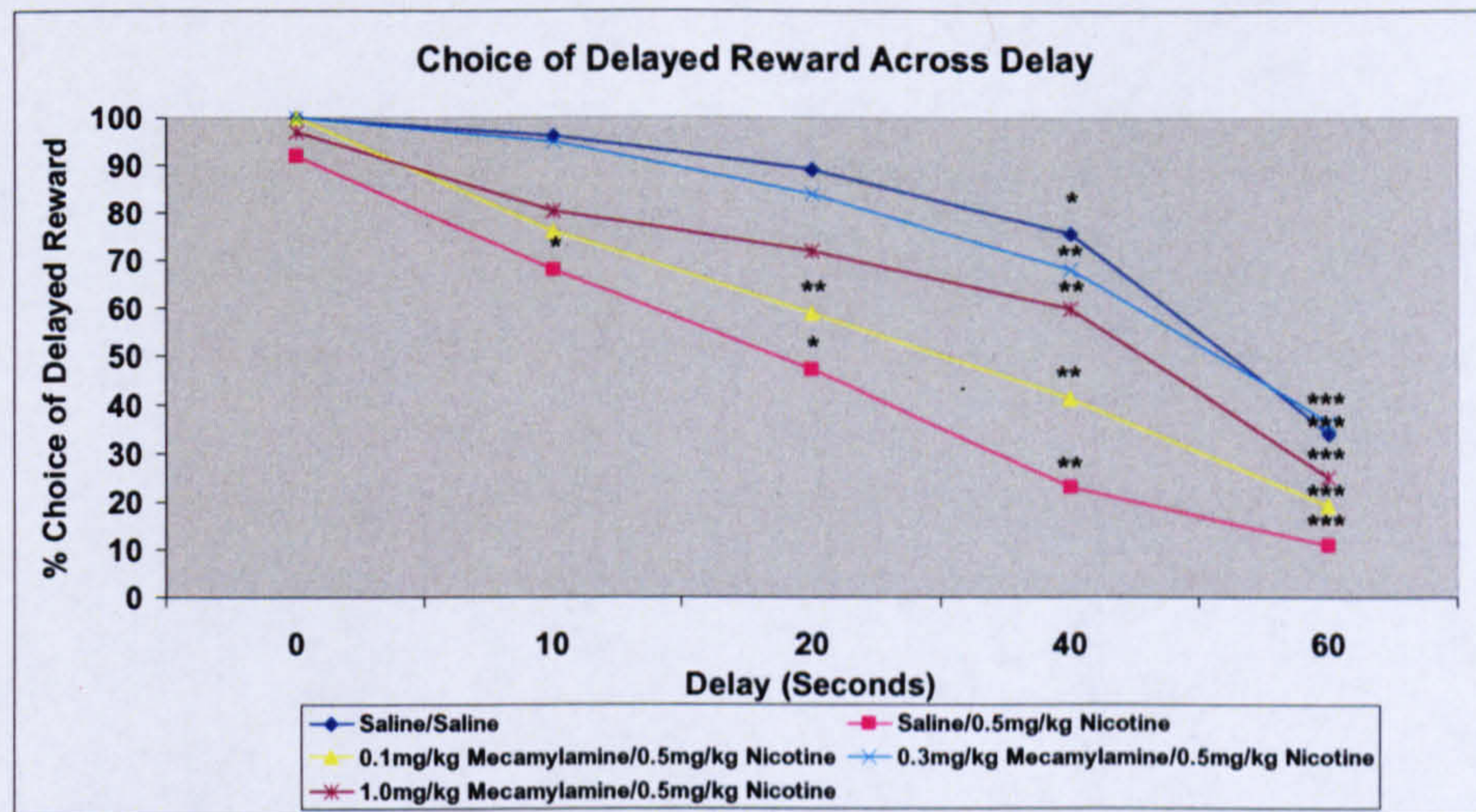


Fig. 7.11: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed at 0 seconds delay.

7.3.5.2. Speed of Responding

As in Experiment 6A, nicotine treatment alone significantly decreased latency to initiate trials in the task in comparison to control ($p < 0.05$) ($F(4,36) = 4.788$, $p = 0.003$). All combination nicotine and MEC treatment doses conversely failed to reach significance in comparison to saline (all $p > 0.05$) (see Fig. 7.12). As shown in Table 7.5 neither nicotine treatment alone or in combination with MEC altered the speed with which animals responded on the lever to select their reward, or the latency with which they collected their reward (all $F(4,36) \leq 2.127$, N.S).

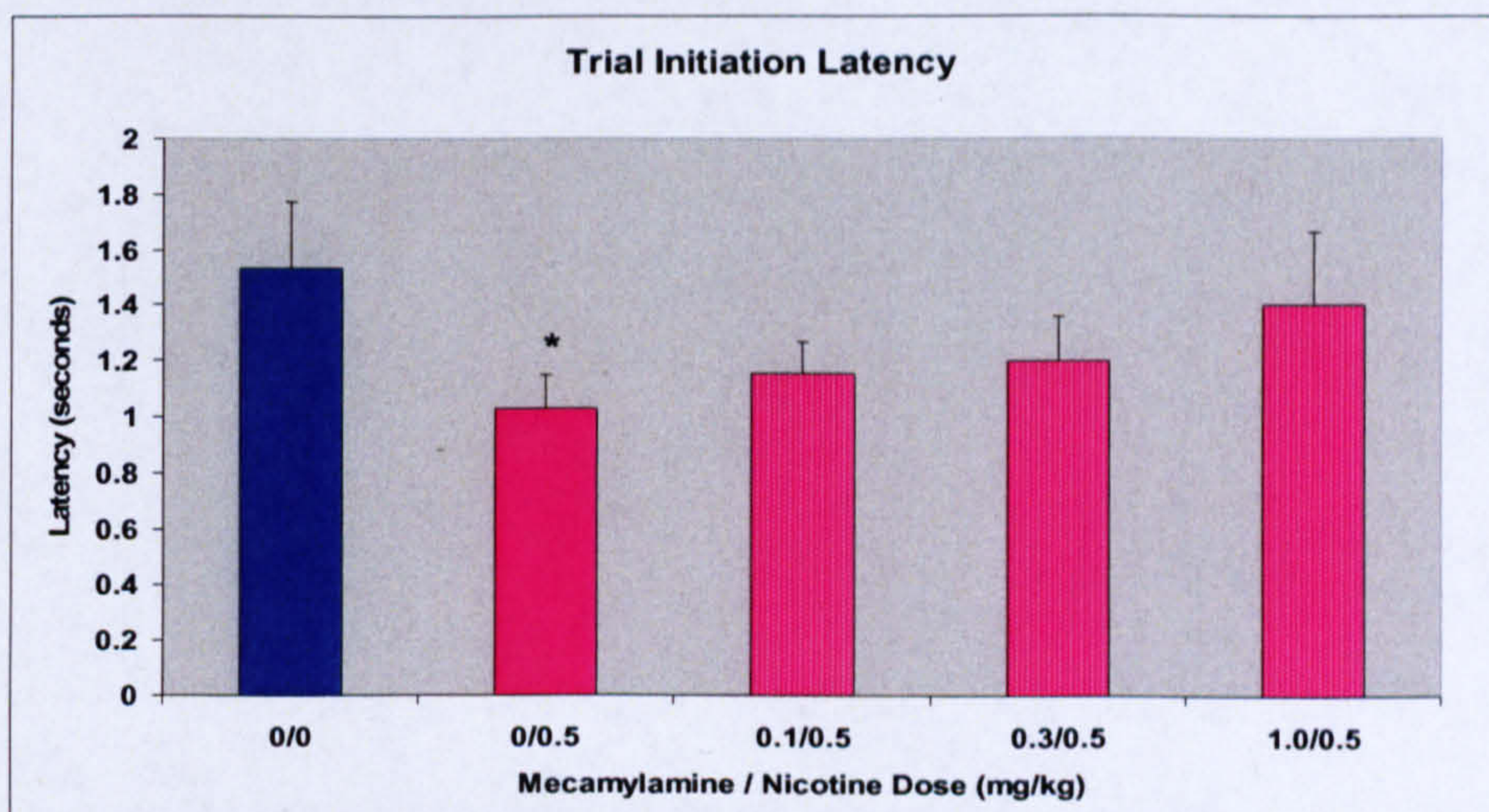


Fig. 7.12: The effects of co-administration of nicotine and mecamylamine on trial initiation latency. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control (0/0mg/kg dose).

7.3.5.3. Omissions

No main effect of treatment was observed on the frequency of trial omissions or failure to collect reward following a delayed reward choice (all $X^2 \leq 7.800$, $df = 4$, N.S) (see Table 7.6).

Table 7.5: The effect of co-administration of nicotine and mecamylamine on speed of responding in the delayed reinforcement task

Behavioural Measure	Mecamylamine Dose (mg/kg) / Nicotine Dose (mg/kg)				
	Saline/ Saline (control)	Saline/ NIC _{0.5mg/kg} (control)	MEC _{0.1mg/kg} / NIC _{0.5mg/kg}	MEC _{0.3mg/kg} / NIC _{0.5mg/kg}	MEC _{1.0mg/kg} / NIC _{0.5mg/kg}
Immediate Response Latency	0.64 ± 0.03	0.73 ± 0.04	0.63 ± 0.03	0.63 ± 0.04	0.63 ± 0.05
Delayed Response Latency	0.71 ± 0.02	0.87 ± 0.11	0.72 ± 0.02	0.72 ± 0.03	0.75 ± 0.05
Immediate Magazine Latency	0.30 ± 0.05	0.27 ± 0.03	0.32 ± 0.06	0.27 ± 0.05	0.26 ± 0.04
Delayed Magazine Latency	1.07 ± 0.35	0.75 ± 0.19	0.75 ± 0.16	1.26 ± 0.47	1.45 ± 0.62

Table 7.5: Each value represents the mean latency ± SEM.

Table 7.6: The effect of co-administration of nicotine and mecamylamine on omissions in the delayed reinforcement task

Behavioural Measure	Mecamylamine Dose (mg/kg) / Nicotine Dose (mg/kg)				
	Saline/ Saline (control)	Saline/ NIC _{0.5mg/kg} (control)	MEC _{0.1mg/kg} / NIC _{0.5mg/kg}	MEC _{0.3mg/kg} / NIC _{0.5mg/kg}	MEC _{1.0mg/kg} / NIC _{0.5mg/kg}
Trial Omissions	0.20 ± 0.13	0.00 ± 0.00	0.30 ± 0.21	0.00 ± 0.00	0.30 ± 0.13
Magazine Omissions Following Delayed Choice	1.80 ± 1.13	0.9 ± 0.64	0.6 ± 0.34	2.7 ± 1.72	1.50 ± 0.87

Table 7.6: Each value represents the mean frequency ± SEM.

7.4. DISCUSSION

The experiments in the present chapter were designed to examine the effects of acute nicotine on impulsive choice and to determine whether these effects were mediated by the central nicotinic receptors. Such research was primarily conducted to elucidate whether the heightened impulsive choice in smokers may in part be a consequence of nicotine exposure, a theory which to date had not been rigorously tested (Bickel et al., 1999; Mitchell, 1999; Baker et al., 2003; Mitchell, 2004; Reynolds et al., 2004a; Reynolds, 2006).

Consistent with the findings of the previous chapter, prior to drug testing animals choice behaviour became contingent on the delay to the delivery of the larger reward. The sensitivity of choice behaviour furthermore remained stable across the 16 weeks of drug testing. The acute administration of nicotine led to a profound dose related decrease in overall choice of the delayed, larger reward. The reduction in choice was also delay dependent. Closer examination of choice behaviour revealed that the effects of nicotine were not due to an alteration in preference of reward during 0 second delay trials. Instead the reductions in choice of delayed reward were indicated at the 10, 20, 40 and 60 second delay trials. With increasing dose of nicotine, the reduction in choice of delayed reward was observed at a greater number of delay trial blocks, supporting further the dose dependent effect of nicotine on choice behaviour (see Figs. 7.3 and 7.4). At the highest 0.5mg/kg dose, selection of the delayed reward was found to be decreased across 10, 20, 40 and 60 second delay trials. Taken together, this pattern of choice behaviour suggests acute nicotine rendered animals substantially more impulsive in their choices (Evenden and Ryan, 1996; Cardinal et al., 2000).

Further support that animals performed more impulsively in the task following nicotine was provided by the more rapid speed at which trials were initiated at the 0.25mg/kg and 0.5mg/kg nicotine doses. Whilst the latency at which the choice between rewards remained unchanged, the speed with which the delayed reward was collected also became significantly faster at the highest dose tested (0.5mg/kg). At this dose, furthermore, a reduction in frequency of omissions to collect the delayed reward was observed.

These findings are consistent with the heightened impulsive choice that has been observed in heavy smokers (Bickel et al., 1999; Mitchell, 1999; Baker et al., 2003; Mitchell, 2004; Reynolds et al., 2004; Reynolds, 2006a), and confirms previous preclinical reports of nicotine induced intolerance to delayed reward in a rodent adjusting delay procedure (Dallery and Locey, 2005). The present results moreover, are in agreement with the increased impulsive choice observed following the acute administration of other drugs of abuse including cocaine, amphetamine and alcohol in comparable delayed reward tasks in both humans and animals (e.g. Logue et al, 1992; Evenden and Ryan, 1996; Cardinal et al., 2000; Hellmans et al., 2005; Helms et al., 2006;

Reynolds et al., 2006).

The notion that nicotine promotes impulsive choice supports the theory that heightened sensitivity to delayed gratification in smokers is in part a consequence of the direct exposure to nicotine. However, other plausible interpretations of the present findings deserve consideration. Firstly, the delayed reward task involves rodents making choices between rewards of both different magnitudes and delay. The increase in choice of the delayed reward following nicotine could therefore be due to either changes in the sensitivity to delay or reward magnitude, or both (Ho et al., 1999). Nicotine treated animals may have selected the immediate reward to a greater degree due to the larger reward not being perceived as valuable as when under control treatment. It is well established in the human literature that when the delayed reward is perceived as less valuable, it is discounted to a greater extent (Green et al., 1997; 1999; Johnson and Bickel, 2002). Going against this argument, however, is that nicotine is known to *increase* the value of sucrose reinforcement (Jias and Ellison, 1990). Furthermore, the majority of research in animals has demonstrated that choice behaviour is less sensitive to changes in reward magnitude than humans (Richards et al., 1997; Grace et al., 1999; Farrar et al., 2003; Green et al., 2004). Finally, nicotine treated animals continued to select the delayed larger reward on almost a 100% of trials during the 0 second delay condition. This suggests that animals were still sensitive to the difference in magnitude of both rewards and able to discriminate effectively between them. Taken together, evidence suggests that the increase preference for the immediate reward is more likely due to nicotine induced hypersensitivity to delay.

An alternative explanation for the observed increase in impulsive choice, is that under drug treatment a response bias or response perseveration on the lever delivering the smaller reward was formed. Once again, the persistent choice of the larger reward during 0 delay trials provides strong evidence against this explanation of the present findings (Mitchell, 2004).

The change in choice behaviour may have instead been governed by nicotine's anorectic effects (McNair and Bryson, 1983; Grunberg et al., 1986; Levin et al., 1987; Blaha et al., 1998; Miyata et al., 1999; Zhang et al., 2001). There is little evidence, however, to support this interpretation. Firstly, in agreement with previous research, the results of the present thesis suggest that decreasing motivation for food reward has no significant effect on levels of impulsive choice in the delayed reward task (Logue and Pena-Correal, 1985; Logue et al., 1988; Cardinal et al., 2000; Wade et al., 2000; Richards et al., 1997). Secondly, if motivation for the reward was reduced it would be predicted that latencies to collect reward in the task would increase. This was not the case; latency to collect delayed reward actually became faster following drug treatment.

Nicotine could instead be affecting cognitive processes argued to play a role in delay discounting tasks, such as working memory. Hinson and colleagues (2003) demonstrated that college students discounted delayed reward more steeply when working memory was impaired by increasing working memory load. Working memory is likely to be a cognitive process involved during the comparison of alternative rewards in the task. As previously discussed, however, nicotine has led to improvements in working memory in both humans and animals (Rusted and Trawley, 2006; Spinelli et al., 2006). Therefore, it is highly unlikely that the observed greater levels of delay discounting can be attributed to disruption in these cognitive processes.

The increased choice of the immediate reward may in part be explained by the known effects of nicotine on time perception (Pradhan and Dutta, 1970; Bizot, 1997; Carrasco et al., 1998). Nicotine is known to increase 'internal clock' speed, i.e. over estimate the passage of time. Under the influence of nicotine, the delay to the delivery of the larger reward is therefore likely to have been perceived as more delayed. As delayed reward is discounted to a greater extent with increasing delay (Green et al., 1994; Bickel and Marsh, 2001), the observed increased preference for the immediate reward may have been mediated by nicotine's induced alterations in timing mechanisms. Providing indirect support for this theory, is the finding that at the highest nicotine dose (0.5mg/kg), latency to collect delayed reward was significantly reduced, as was the frequency of failures to collect delayed reward. These data could indicate that the animal had over estimated the passage of time during the delay to the delivery of reward, and therefore approached the magazine to await reward delivery at an earlier time period. Being at a closer proximity to the magazine when the reward was delivered would have consequently led to faster magazine latencies and decreased likelihood of magazine omissions. This theory is however tentative. In order to have supported more accurately the disruption in timing ability in the task, a measure of magazine entries during the period of delay would have been more indicative of this effect. Unfortunately, this measure was not taken. Despite the present research being unable to conclusively determine whether an overestimation of the passage of time mediated the increase in preference for immediate reward, from the reviewed evidence it appears highly likely that there is a close association between time perception and delay discounting.

Taken together, the increased preference of the immediate reward appears most likely to have been mediated by the nicotine induced hypersensitivity to delay, leading to a genuine increase in impulsivity. It appears however, that time perception may be a closely related process to delay discounting. Nicotine, therefore, may in part have increased delay sensitivity via its influence on timing mechanisms.

Experiment 6B investigated the effects of the centrally acting nicotinic antagonist, MEC, on levels of impulsive choice. The antagonist alone displayed no effect on overall percentage choice of delayed reward in comparison to control treatment. Evidence of greater delay dependency of choice behaviour was however indicated following the highest MEC dose (1.0mg/kg). At this dose choice of delayed reward was significantly reduced across a greater number of delay trial blocks (20, 40 and 60seconds) in comparison to choice behaviour at the zero delay condition. This was in contrast to control treatment where choice behaviour only differed at 40 and 60 second delay conditions. It is important to consider, however, that preference for the larger reward did not differ significantly between the highest MEC dose and saline treatment across all delay conditions. Other parameters in the task, including speed of responding and frequency of omissions also remained unchanged following MEC administration.

When co-administered with 0.5mg/kg nicotine, 0.3mg/kg MEC antagonised the nicotine induced hypersensitivity to delay. At the MEC_{0.3mg/kg}/NIC_{0.5mg/kg} dose, overall choice of delayed reward, and choice by delay did not differ from saline treatment, supporting the complete reversal of nicotine's effects on impulsive choice. Conversely, co-administration of nicotine with 0.1 and 1.0mg/kg MEC failed to block nicotine's effects on choice behaviour, with both combination doses reducing choice of delayed reward significantly in comparison to control. Furthermore, analysis of choice by delay revealed that preference of delayed reward was specifically reduced at 20 second delay trials in comparison to saline treatment at both the MEC_{0.1mg/kg}/NIC_{0.5mg/kg} and MEC_{1.0mg/kg}/NIC_{0.5mg/kg} doses. Replicating the effects on task performance in Experiment 6A, nicotine alone once again significantly decreased latency to initiate trials. In contrast to impulsive choice this effect was reversed by co-administration of all MEC pre-treatment doses.

These findings suggest that an optimal dose of MEC is required to block nicotine's effects on choice behaviour; indicating perhaps evidence of competitive antagonism of the effects of nicotine on impulsive choice, via blockade of the centrally located nicotinic receptors. Although the classic antagonist is generally believed to be non-competitive in its action, it has reported previously that MEC may also exhibit some competitive properties, for example on behaviours such as locomotor activity (Varanda et al., 1985; Martin, 1989; 1990; Francis and Papke, 1996). The studies of the present chapter are the first of preclinical research to demonstrate that nicotine's effect on impulsive choice are mediated by the central nAChRs. However, the lack of selectivity of MEC does not enable elucidation of the specific nAChR subtypes that may be involved in this effect of nicotine (Chavez-Nriega et al., 1997). As previously discussed in Chapter 4, research has implicated the $\alpha 4\beta 2$ and $\alpha 7$ receptors in the mediation of nicotine

induced disinhibition (Blondel et al., 2000; Grottick and Higgins, 2000; Keller et al., 2005; Hoyle et al., 2006). Whether these receptor subtypes are additionally involved in the modulation of impulsive choice is unknown. Extensive research is available to suggest, however, that the subcomponents of the impulsivity are often dissociated at the receptor subtype level, as is the case with both serotonergic and dopaminergic systems (Wade et al., 2000; Talpos et al., 2006; van Gaalen et al., 2006). Through the use of more selective nicotinic antagonists, such as DH β E, future research should focus upon dissociating the role of specific nicotinic subtypes in mediation of impulsive choice.

The neurochemical events beyond the nicotinic receptor were not investigated in the present research. However, taking together the present findings of the nicotine induced impulsivity with previous observations of the increase in impulsive choice following both cocaine and amphetamine, suggests that a common neurobiological mechanism is likely to be involved (Logue et al., 1992; Evenden and Ryan, 1996; Cardinal et al., 2000; Helms et al., 2006). One possible candidate is therefore the increase in dopaminergic neurotransmission resulting from nicotine administration (Di Chiara and Imperato, 1988). Nicotine, via stimulation of the nAChRs located within the VTA, leads to the enhancement of extracellular levels of DA in the NAc and areas of PFC cortex (Oades and Halliday, 1987; Di Chiara and Imperato, 1988; Benwell and Balfour, 1992; Nisell et al., 1994; 1996; Cadoni and Di Chiara, 2000). As both the NAc and orbitofrontal region of PFC have long been implicated in the neuronal processes underlying impulsive choice, it is reasonable to argue that the alteration in DA neurotransmission in these regions are likely to play role in the nicotine induced intolerance to delayed reward (Cardinal et al., 2001; Kheramin et al., 2002; 2004 Mobini et al., 2002; Winstanely et al., 2004; Pothuizen et al., 2005).

This is not to ignore the fact that nicotine is also known to stimulate the release of several other neurotransmitters, including noreadrenaline, 5-HT, glutamate and GABA (Wonnacott et al., 1990; Wonnacott, 1997; Li et al., 1998; Jones et al., 1999), that may also be involved in the acute pharmacological effects of nicotine on discounting of delayed reward. Whilst there is extensive support for the role of 5-HT in the neurobiology of impulsive choice, there currently is limited evidence available of the potential involvement of other neurotransmitter systems (e.g. Wogar et al., 1993; Ho et al., 1998; Mobini et al., 2000a; 2000b). Future studies should identify conclusively the neuronal processes involved in the association between nicotine and intolerance to delayed reward. Such data will prove crucial if impulsive choice is to become a target for future pharmacological treatment strategies for nicotine addiction.

7.4.1. Conclusions

In summary, the findings of the present chapter have demonstrated that acute nicotine induces a

profound reduction in choice of delayed that is most likely governed by the increased sensitivity to delay. This effect moreover appears to be mediated by the central nicotinic receptors. These findings suggest that the initial exposure to nicotine in drug naïve individuals may render them hypersensitive to delay. This may result in the longer term social and health benefits, associated with a continued drug free lifestyle, to be highly discounted at this stage. As a consequence, individuals may more likely select the immediate reinforcing properties of nicotine, thereby leading to continued smoking and likelihood of the development of dependence.

CHAPTER 8

The effects of chronic nicotine administration and nicotine withdrawal on impulsive choice

8.1. INTRODUCTION

8.1.1. General Introduction

Experiment 7 demonstrated that when administered acutely, nicotine leads to a profound increase in impulsive choice, as supported by the delay dependent reduction in choice of the delayed larger reward. In Chapter 5 it was proposed that in order to gain a more in depth understanding of the role played by impulsivity in the maintenance and relapse of drug addictive disorders, it is important that the effects of chronic nicotine, nicotine withdrawal, and the alterations in responsivity to nicotine following a period of abstinence, are additionally explored on aspects of impulsivity.

To date, only one study has explored the effects of chronic nicotine treatment on the sensitivity of delayed gratification. A comparison was made of the effects of acute nicotine challenges administered prior to and during the final stages of 65 days of chronic nicotine treatment. During the 65 day period, rats were treated daily with a single 0.3mg/kg injection of nicotine. Findings demonstrated that greater levels of discounting of delayed reward were exhibited following the chronic exposure to nicotine, including treatment of the saline dose (Dallery and Locey, 2005). However, as previously discussed, the methodological limitations of Dallery and Locey (2005) study, such as the considerably low subject number (N=5), raises issues regarding the validity of findings. The reported heightened impulsive choice following chronic nicotine exposure is nevertheless consistent with the increase in impulsivity observed during the repeated administration of methamphetamine (Richards et al., 1999a) and cocaine (Logue et al. 1992; Paine et al., 2003) in comparable rodent models of delayed reward.

To determine the contribution of impulsive choice to the risk of relapse in smokers, it is important to assess the levels of sensitivity to delayed gratification during the first two weeks of withdrawal when smokers are known to be at the greatest risk of relapse (Gossop et al., 1989; Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995). Indeed, smokers when nicotine deprived have been found to discount both hypothetical monetary rewards (Field et al., 2006) and cigarettes (Mitchell et al., 2004; Field et al., 2006) to a significantly greater extent in comparison to when nicotine sated. Longer term abstinence from

nicotine, has in contrast, been associated with a reduction in levels of delay discounting that a more comparable to the rates of non-smokers (Bickel et al., 1999). Taken together, these findings suggest perhaps a biphasic relationship between abstinence and impulsive choice may exist. However, the lack of information regarding pre-smoking levels of impulsive choice in these individuals hinders interpretation of the relationship between abstinence and levels of delay discounting. It is possible, that initially drug deprivation leads to an increased rate of discounting of delayed reward, but is then followed with a decrease in impulsive choice with continued abstinence. Conversely, the reductions in delay discounting with longer term abstinence could suggest that lower levels of impulsivity may have enabled these individuals to successfully abstain from smoking (Bickel et al., 1999). In support of the latter theory, more recent research had demonstrated that higher levels of impulsive choice on behavioural delayed reward tasks significantly predicted smoking relapse (Krishan-Sarin et al., 2007; Yoon et al., 2007). Only longitudinal research that assesses the level of discounting at the point of cessation through to longer term abstinence will enable the establishment of the relationship between impulsive choice and smoking abstinence.

Smokers, as with other drug abusers are known to relapse long after drug withdrawal (Stephens and Cottrell, 1972; Robinson and Berridge, 2000; 2003; Hughes et al., 2004; Piasecki, 2006; Hser, 2007). The mechanisms involved in long term drug relapse, are currently not well understood. Whilst theories have implicated a loss of inhibitory as a key component in drug relapse (Jentsh and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004), the roles of other aspects of impulsivity, such as impulsive choice, have not been thoroughly investigated. Chapter 5 of this thesis demonstrated that chronic drug exposure can render animals hypersensitive to the effects of nicotine on disinhibition. These effects were observed long after cessation of drug treatment and when levels of inhibition had returned to BL. These findings present strong evidence of the key role of this aspect of impulsivity in drug relapse, and may in part explain why a single lapse in smoking is one of strongest predictors of relapse (Brandon et al., 1990; Nides et al., 1995; Shiffman et al., 1996). Whether a comparable increase in responsivity to the effects of nicotine on impulsive choice is evident is currently unknown. Prolonged exposure to nicotine, however, is known to lead to alterations in serotonergic and dopaminergic systems in regions implicated in impulsive choice, including the VTA and its associated projections the NAc and OPFC (e.g. Kirch et al., 1987; Vezina et al., 1992; Ramussen and Czachura, 1995; Ramussen and Czachura, 1995; Nisell, 1996; Balfour et al., 1998; Hildebrand et al., 1998; 1999; Mobini et al., 2000a; Wade et al., 2000; Olausson et al., 2001; Rahman et al., 2004; Winstanley et al., 2004; Pothuizen et al., 2005). These neural adaptations may also increase the sensitivity to the effects of nicotine on impulsive choice. If this is the case, smoking during cessation may lead to the delayed rewards associated with continued abstinence to be discounted to a greater degree. This in turn may lead to individuals

selecting once again the more immediate rewarding effects of nicotine (relapse).

8.1.2. Objectives of Experiment 7 and 8

In an attempt to gain further understanding of the complex association between nicotine dependence and impulsive choice the objectives of Experiments 7 and 8 were therefore:

- i) To determine the effects of chronic nicotine exposure on impulsive choice as measured by the delayed reward task (Experiment 7).
- ii) To assess changes in levels of impulsive choice during the early stages of withdrawal when smokers are particularly vulnerable to relapse (Experiment 7).
- iii) To assess the stability of changes in impulsive choice during longer term abstinence (Experiment 7).
- iv) To establish whether chronic drug exposure and withdrawal leads to long term alterations in responsivity to the effects of acute nicotine administration on impulsive choice (Experiment 8).
- v) To preliminarily explore differences in response of high and low “trait” impulsive animals to the effects of chronic nicotine treatment, nicotine withdrawal and acute nicotine following longer term abstinence (Experiment 7 and 8).

To achieve these objectives a longitudinal study was conducted in Experiment 7 during which the levels of impulsive choice of subjects was assessed prior to the initiation of drug treatment, during seven days of chronic nicotine administration, and during initial and long term nicotine withdrawal. The same subjects were then tested by a series of acute nicotine challenges in Experiment 8. Comparable to Chapter 5, in order to ensure that animals were experiencing nicotine withdrawal, assessment was additionally made of the nicotine abstinence syndrome (Malin et al., 1992).

The findings of Chapter 6 indicated that marked individual differences in sensitivity to delayed gratification at baseline were observed, possibly reflecting differences in “trait” level of impulsiveness (Winstanley et al., 2003; Deltu-Hagedorn 2006). Whilst research has demonstrated that high levels of impulsive choice can lead to greater self administration of cocaine (Perry et al., 2005) and consumption of alcohol (Poulos et al., 1995), it has yet to be determined whether high and low impulsive animals respond differentially to the effects of nicotine on impulsive choice. To therefore achieve the final objective of this chapter, individual differences in levels of impulsive choice were determined through the application of the exponential delay discounting function to BL choice data. The differences in response of high and low impulsive animals to the effects of chronic nicotine, nicotine withdrawal and residual sensitivity to nicotine following a period of abstinence were then explored. As this was only a

preliminary investigation with a small number of subjects, the effects were only assessed on the primary measure of impulsive choice.

8.2: EXPERIMENT 7: ASSESSMENT OF THE EFFECTS OF CHRONIC NICOTINE ADMINISTRATION AND NICOTINE WITHDRAWAL ON IMPULSIVE CHOICE

8.2.1. METHOD

8.2.1.1. Subjects

Subjects were 24 adult male Lister Hooded rats (Charles River, UK). Animals were housed in pairs on arrival at the laboratory and maintained under a 12 hour light/dark cycle (lights on at 0700h) at controlled environmental temperature of $21\text{ }^{\circ}\text{C} \pm 4^{\circ}\text{C}$ and relative humidity of $50\% \pm 10\%$. During experimentation food was restricted in order to maintain animals at 85% of their free feeding body weights. Water was available ad libitum in home cages and feeding occurred at the end of each experimental day. At the start of testing, animals weighed approximately 360-380g. All animals were treated in accordance with the UK Animals (Scientific Procedures) Act 1986.

8.2.1.2. Apparatus

Subjects conducted the delayed reinforcement task in two sets of four operant chambers (dimensions 30.5 X 24.1 X 21cm and 30.5 X 24.1 X 29.2cm Med Associates Inc., USA). During the observation of somatic withdrawal signs animals were placed in a glass observation tank (dimensions 40.5 X 37 X 11 cm). Behaviours were recorded by a video camera (JVC TK-128OE) positioned horizontally in front of the observation chamber. The camera was relayed to a monitor (TM-1500PS) in an adjacent laboratory. For a more detailed description of all apparatus refer to Chapter 2 section 2.5.1 and 2.6.1.

8.2.1.3. Behavioural Testing

The behavioural procedure of the delayed reward task, in addition to the pre-training required has been described previously in detail in Chapter 6, section 6.2.1.3. The behavioural measures assessed in the paradigm are also addition detailed in this section. Training continued on the task until subjects displayed choice behaviour that was delay sensitive and stable across a ten day period. Animals completed one session of the task per day and training was completed within approximately 10 weeks.

8.2.1.4. Drugs

Nicotine hydrogen tartrate salt was dissolved in 0.9% saline and the pH adjusted to approximately 6, using 0.1M and 1.0M sodium hydroxide. The drug solution was chronically administered through s.c. osmotic mini pumps (Model 2ML1; Alzet, Charles River, UK) and the concentration calculated so that animals received 3.16mg/kg/day for a seven-day duration.

Nicotine was released at a rate of 10µl/hr (see also section 2.3.1).

8.2.1.5. Experimental Design and Procedure

A mixed design was employed to examine the chronic effects of nicotine on impulsive choice, with treatment group (chronic saline (n = 11) or nicotine treatment (n = 13)) as the between subject factor and test day as the within subject factor. Animals were separated into treatment group prior to the implantation of osmotic pumps, matched for baseline levels of impulsive choice. All experimentation followed standard operant testing procedures, outlined in detail in the general methodology of Chapter 2, section 2.5.2. All operant testing took place during the light phase of their LD cycle between 0830h and 1730h.

8.2.1.5.1. The Effects of Chronic Nicotine Administration and Nicotine Withdrawal on Impulsive Choice in the Delayed Reward Task

Once trained, animals were separated into two treatment groups and their base line behaviour assessed in the operant task across a seven day period. Following the examination of BL behaviour, osmotic pumps were then surgically implanted under isoflurane/oxygen anaesthesia, and dependent on which group the animals were allocated to, filled with either saline or nicotine. For a more detailed description of surgical procedures refer to the general thesis methodology of Chapter 2, section 2.4. Forty two hours following the implantation of the osmotic pumps (or thirty six hours following the initiation of drug release) the effects of the chronic administration of nicotine on impulsive choice were measured. For the duration of the forty two hours post surgery animals remained in their home cage to allow them to recover from surgery prior to operant testing. During the seven days of drug administration, behaviour in the delayed discounting task was examined during six sessions, with animals conducting the task once per day.

The initiation of spontaneous nicotine withdrawal was then achieved through the removal of the pumps on the seventh day following pump implantation. Examination of performance in the delayed discounting task began 12 hours following pump removal and continued for a three week period. During this time animals conducted one operant test session per day.

8.2.1.5.2. Assessment of the Nicotine Abstinence Syndrome

The intensity of spontaneous withdrawal was assessed by observing the frequency of somatic signs. This was conducted using a nicotine abstinence scale developed by Malin et al., (1992). Briefly the number of somatic signs, including gasps, writhes, body shakes, head shakes, chews, teeth chattering, cheek tremors, paw tremors, genital grooming, foot licks, yawns, ptosis and scratches were recorded. A detailed description of these behaviours are summarised in the general methodology of Chapter 2, section 2.6.2 and Table 2.1.

Prior to assessment, all animals were habituated to both the test room and observation chamber on two consecutive days. Somatic signs were observed, the day before pump implantation (baseline assessment), the final day of chronic drug infusion, and during the first week following the termination of drug treatment. During withdrawal somatic signs were recorded at 6, 13.5, 18, 24, 37.5, 61.5, 85.5, 109.5, 133.5, 157.5 and 181.5 hours following pump removal. The majority of somatic signs were assessed by the experimenter immediately following operant behavioural testing for a period of ten minutes. The exception to this was during the early onset of withdrawal, when somatic signs were also observed at 6, 18 and 24 hours when operant testing did not take place. The time points of observational sessions were selected on the basis previously reported elevation in somatic signs during nicotine withdrawal (Malin et al., 1992; Malin et al., 1994; Hilderbrand et al., 1997; Epping-Jordan et al., 1998; Harrison et al., 2001).

8.2.1.6. Statistical Analysis

In the case of all statistical procedures data prior to analysis were assessed for normality and transformed where necessary (see also section 2.7). Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction. The homogeneity of variance of between subject variables was assessed by Levines test. In all cases of analysis α values of $p < 0.05$ were deemed statistically significant.

8.2.1.6.1. The Effects of Chronic Nicotine Administration and Nicotine Withdrawal on Impulsive Choice in the Delayed Reward Task

Comparison of performance on the task by each treatment group was analysed across each stage of treatment; including baseline, chronic drug administration, withdrawal week one, withdrawal week two and withdrawal week three. Data from seven operant sessions were examined for each stage with the exception of chronic drug treatment where only six test sessions were conducted.

To assess differences in performance between treatment groups during baseline, a two-way mixed ANOVA was conducted on all behavioural parameters (CDR and speed of responding), with treatment group (nicotine or saline) as the between subject factor and test day as the within subject factor. Comparison of choice of reward by delay, involved a three-way mixed ANOVA being applied to the data, with treatment group as the between subject factor and both test day and delay (0, 10, 20, 40 and 60s) as the within subject variables. All significant main effects were analysed further by Bonferroni post hoc comparisons and interactions were followed where appropriate by simple effects analysis. Single repeated measures ANOVA analysed the stability of behaviour across test days for each treatment group. Independent t-tests analysed differences between each group on measures at individual test days (Bonferroni correction of $p <$

0.007).

The data gathered during BL week was, furthermore, assessed to determine the classification of animals as either a high or low impulsive subject. This was achieved by applying an exponential curve to the data for each subject to determine the degree of discounting of delayed reward (see Chapter 6, section 6.2.1.5.2. for more detail on statistical procedure).

Both within and across treatment groups, animals demonstrated extensive variability in BL performance across parameters within the task. To control therefore for the possibility that pre-drug differences in behaviour may influence the dependent variable being measured during both **chronic** and **withdrawal** treatment stages, all data was subjected to a two-way mixed ANCOVA (Howell et al., 1992; Stevens 1992; Tabachnick and Fidell 2007). At each treatment stage behavioural parameters were assessed with the average performance under baseline conditions acting as the covariate within the model. This analysis enabled pre-drug differences in baseline behaviour to be controlled for, thus reducing error variance and allowing a more accurate assessment of the effects of chronic drug treatment and withdrawal on behaviour. ANCOVA was applied to variables measuring overall CDR and speed of responding. Due to the complex design, choice of the larger reward across delays was independently assessed at each of the five delays. Analysing choice data in such a manner enabled baseline choice of delayed reward to be controlled for within the ANCOVA at each delay. For all conducted ANCOVAs, treatment group represented the between subject factor, whilst test day the within subject factor. Significant main effects were explored further by post hoc Bonferroni tests. Significant treatment group x test day interactions were examined further by simple effects analysis; where individual means compared were adjusted to control for the effects of the covariate. One-way repeated measures ANCOVAs examined by group the stability of behaviour across test days, whilst a univariate ANCOVA compared groups at each individual test day (drug week, Bonferroni correction of $p < 0.008$; withdrawal week 1-3, Bonferroni correction of $p < 0.007$).

Prior to all ANCOVA a further assumption assessed was that of homogeneity of regression. This was assessed via the examination of interactions of the covariate with both the within and between subject variables. If homogeneity of regression was found to be violated (i.e. interactions were found to be significant), then the use of ANCOVA was no longer appropriate. Though rare, in such instances data was expressed instead as a percentage change from baseline and analysed by a two-way mixed ANOVA (Tabchnick and Fidell, 2007).

To assess finally whether baseline differences in impulsive choice affected animals response to the chronic administration and withdrawal from nicotine, data from high and low impulsive animals were analysed independently and compared. The application of exponential curves to

baseline data identified a group of low impulsive subjects allocated across treatment groups (nicotine group ($n = 9$); saline group ($n = 6$)). A smaller group of highly impulsive animals were in addition present within each treatment condition (nicotine group ($n = 4$); saline group ($n = 5$); see result section 8.5.2.6). The examination of treatment stage on impulsive choice in low impulsive animals was assessed as above. Due to the low sample size of highly impulsive animals, data was not subjected to statistical analysis; descriptives of choice behaviour were instead plotted and explored.

8.2.1.6.1. Assessment of Somatic Withdrawal Signs

Total somatic signs were analysed using a two-way mixed factors ANOVA, with treatment group as the between subject factor and observational session as the within subject factor. Bonerrfoni post hoc comparisons were applied to the data following significant main effects, whilst one-way ANOVA and independent t-tests were used to examine further significant interactions. As normality was violated, individual categories of withdrawal symptomology were, in contrast, analysed non-parametrically (see Table 2.1). Examination of somatic signs by treatment group across sessions were analysed by Freidman and Wilcoxon tests, while comparison between groups across sessions were performed by Mann Whitney procedural tests.

8.2.2. RESULTS

Figure 8.1 illustrates and compares the overall percentage choice of delayed reward across all stages of study. Fig. 8.2 (a-e) illustrate choice behaviour by delay. The following results sections of this chapter will explore in sequence the differences in performance on the delayed reward task between treatment groups across stages of baseline, chronic drug treatment and withdrawal weeks one, two and three. All graphs and tabulated data represent actual mean values.

Across all ANCOVA of performance during both drug treatment and withdrawal the effect of the covariate (average baseline performance) remained highly significant for all parameters measured within the task (all $F(1,21) \geq 20.409$, $p \leq 0.001$). This indicated the importance of controlling for BL differences in behaviour within analysis. With the exception of the magazine latency, following an immediate reward choice, homogeneity of regression was satisfactory for all measures within the task including both choice and speed of responding parameters. Across behaviours within the task no significant interactions were displayed between the covariate and the between subject factor of treatment group (all $F(1,20) \leq 0.001$, N.S) or within subject factor of test day (drug week: all $F(5,100) \leq 0.185$, N.S ; withdrawal week one : all $F(6,120) \leq 0.222$, N.S ; withdrawal week two : all $F(6,120) \leq 0.290$, N.S; withdrawal week three : (all $F(6,120) \leq 0.443$, N.S.). Homogeneity of regression was however violated for immediate magazine latency across stages of study, with a significant interaction shown between the covariate and within

subject factor of test day (drug week: $F(5,100) = 6.541, p < 0.001$; withdrawal week one : $F(6,120) = 6.697, p < 0.001$; withdrawal week two : $F(6,120) = 11.770, p < 0.001$; withdrawal week three : $F(6,120) = 5.547, p = 0.003$). The covariate displayed no interaction however with the between subject factor of treatment group on this measure (all $F(1,20) \leq 0.039, N.S$) This speed of responding measure was therefore expressed as a percentage change from BL and analysed by a two-way mixed ANOVA.

Analysis of behaviour during both BL, chronic drug administration and withdrawal, unless otherwise stated, displayed no significant main effects of test day on measures of choice behaviour or speed of responding within the delayed reinforcement task. The failure to initiate trials, choose a reward and to collect the reward following both immediate and delayed reward selection rarely occurred across all treatment stages. Analysis of these parameters was therefore not conducted.

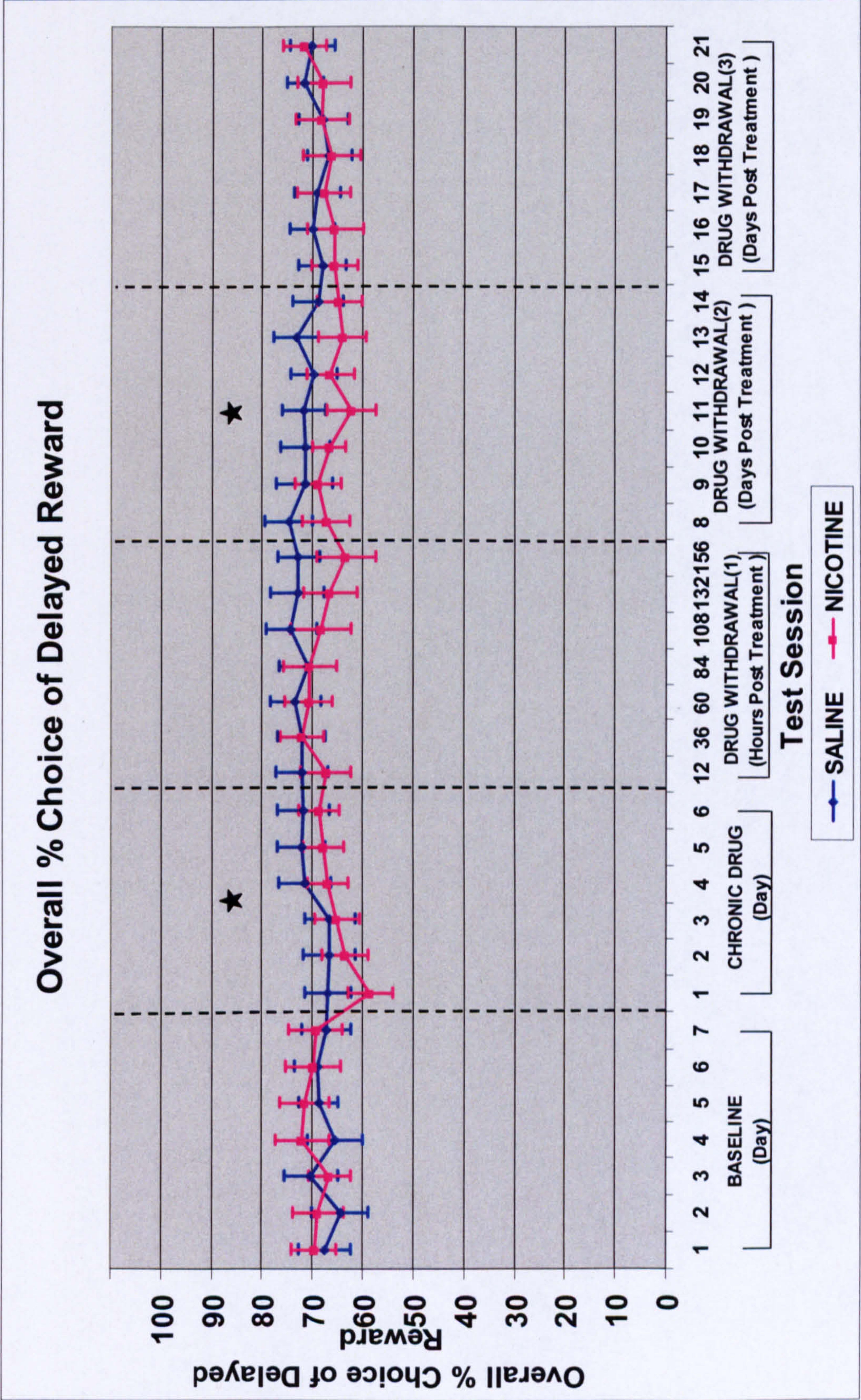


Fig. 8.1: Choice Behaviour: the effects of chronic nicotine administration and withdrawal on overall percentage choice of delayed reward. Each point represents the mean percentage choice.

★ $p < 0.05$ Main effect of group

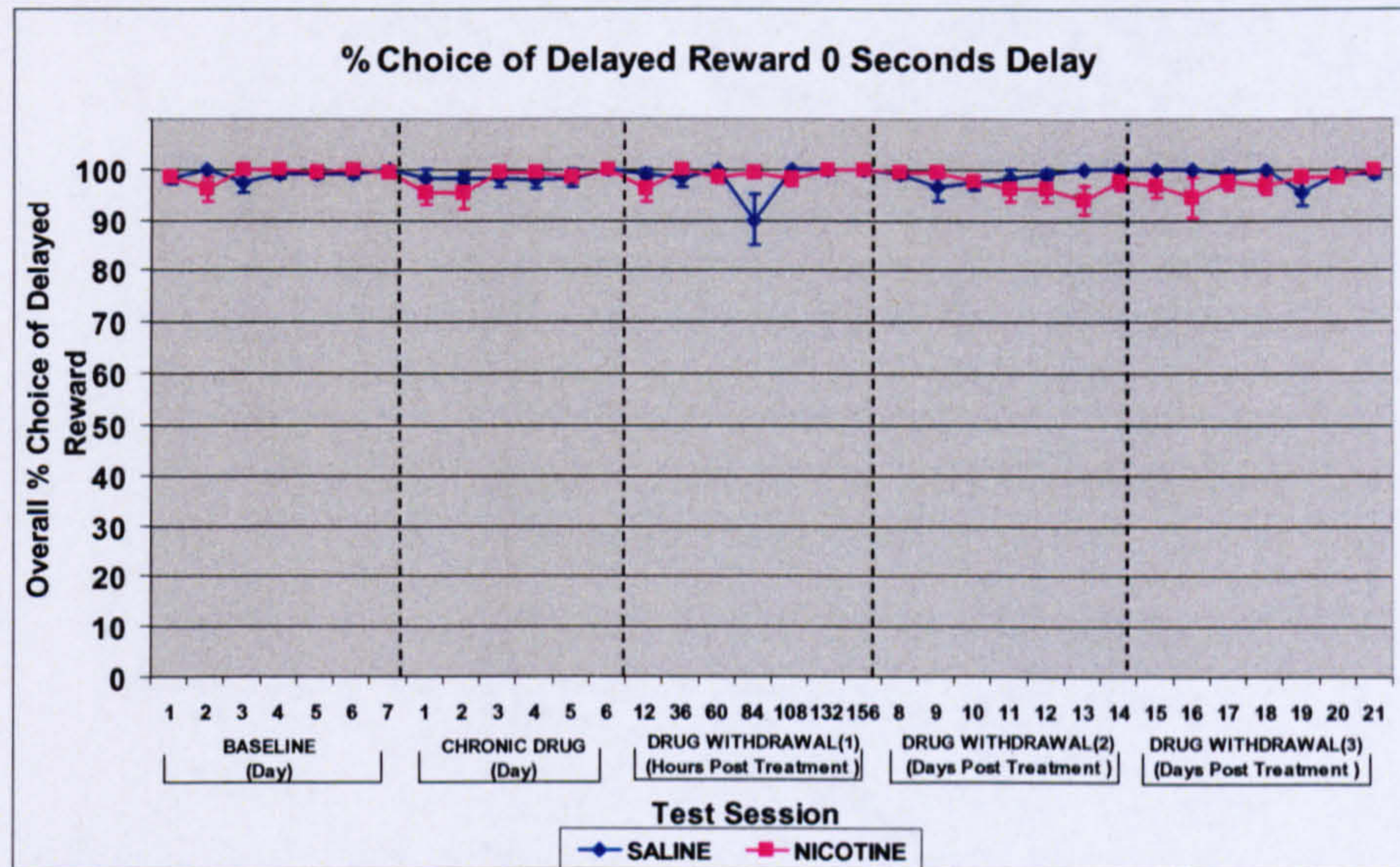


Fig. 8.2 (a)

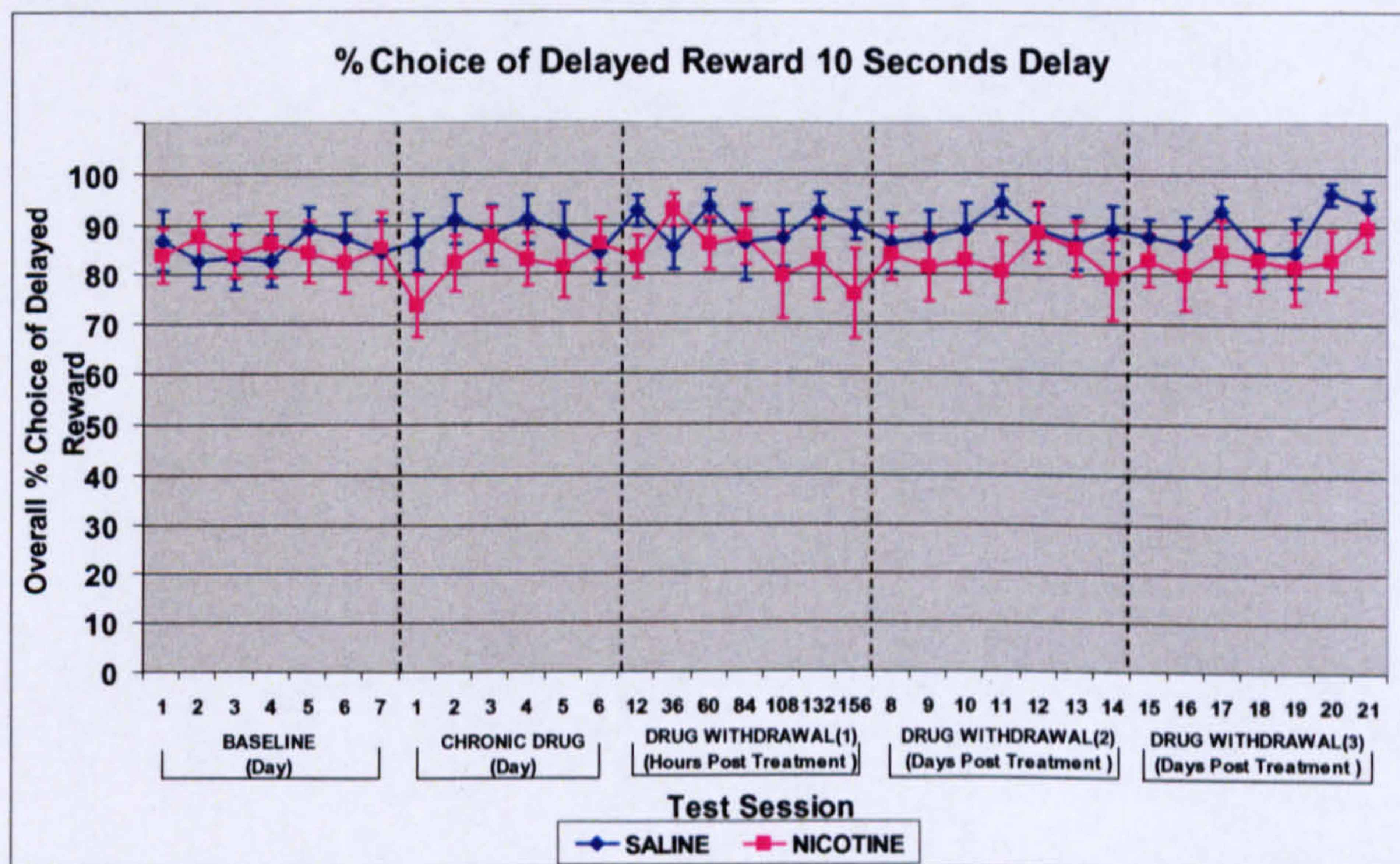


Fig. 8.2 (b)

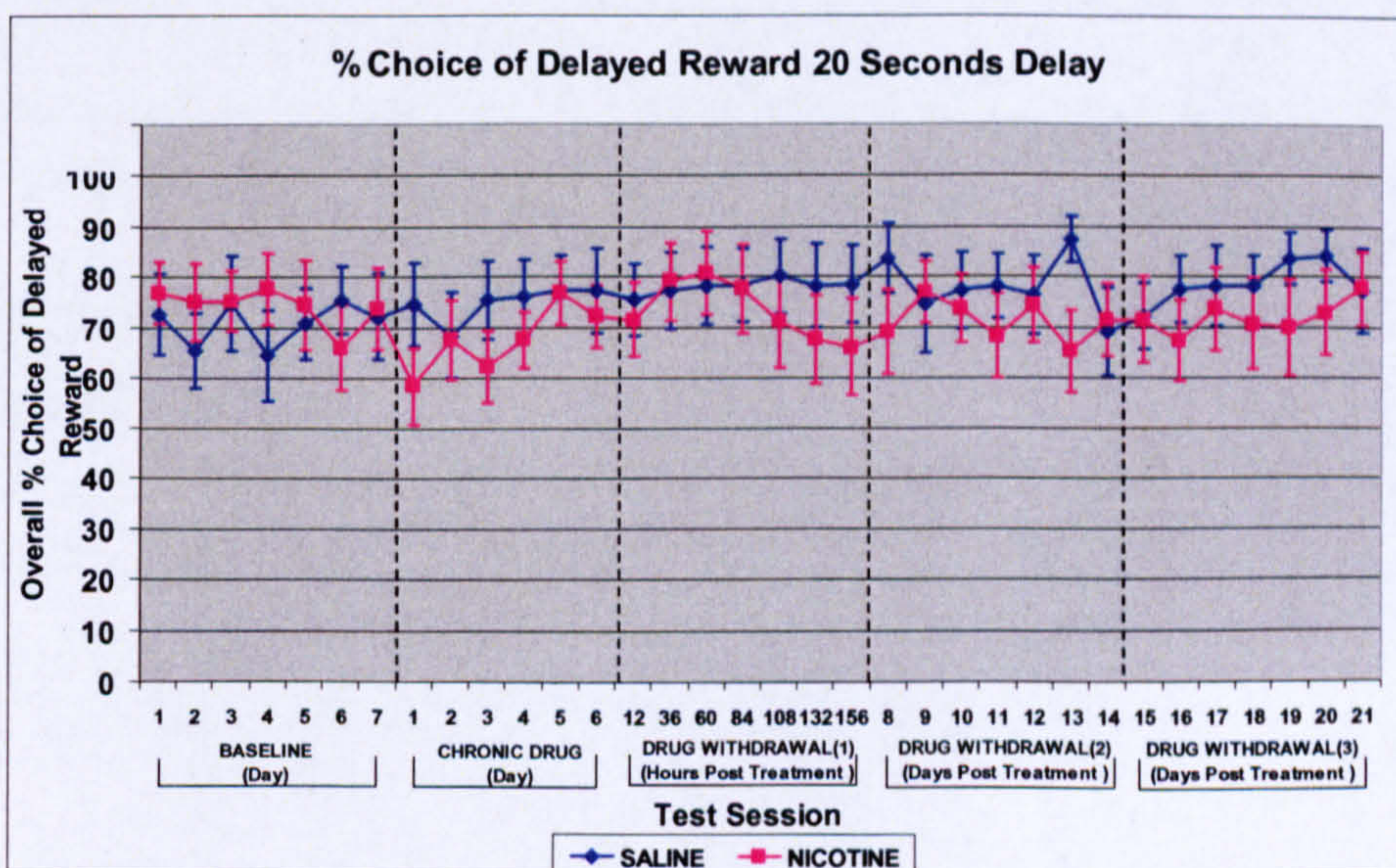


Fig. 8.2(c)

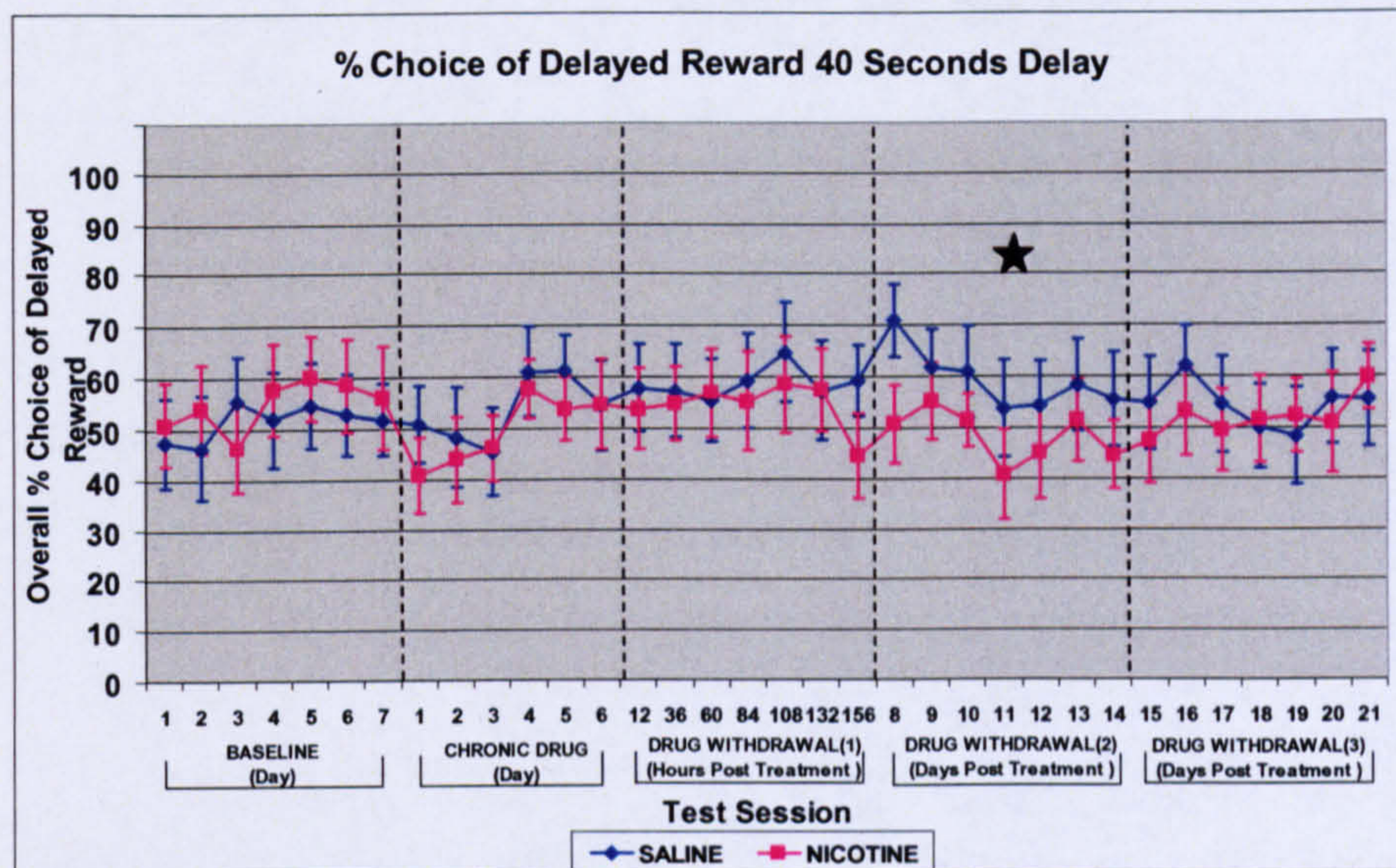


Fig. 8.2 (d)

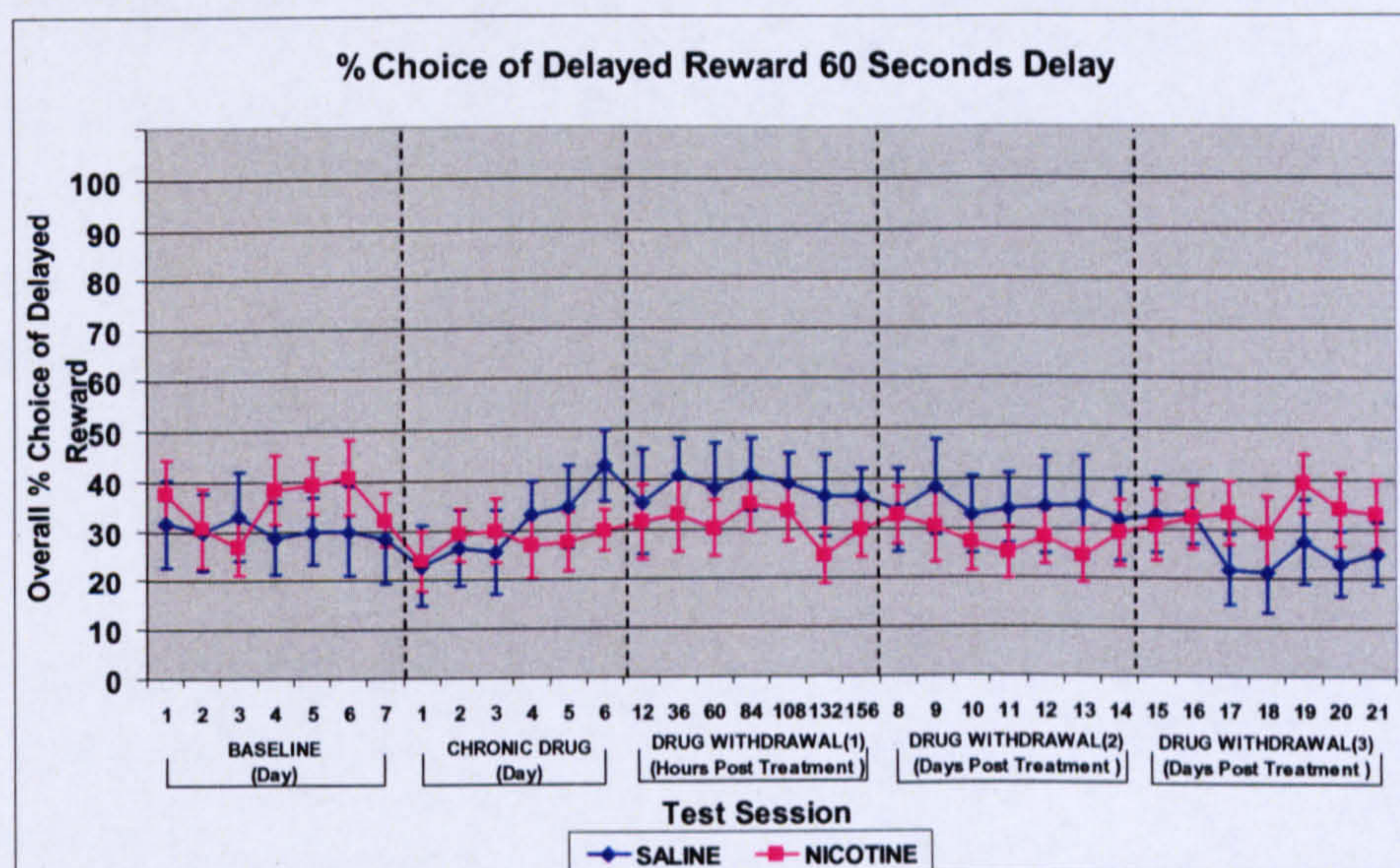


Fig. 8.2 (e)

Fig. 8.2 (a-e): Choice Behaviour: the effects of chronic nicotine administration and withdrawal on percentage choice of delayed reward during each delay. Each point represents the mean percentage choice. ★ $p < 0.05$ Main effect of group.

8.2.2.1. Baseline

8.2.2.1.1. Choice Behaviour

Prior to chronic drug treatment overall choice of delayed reward was stable, as indicated by the non-significant main effect of test day ($F(6,132) = 0.723$, N.S.). Choice behaviour was comparable across treatment groups as shown by the non-significant main effect of group ($F(1,22) = 0.095$, N.S.) and group \times test day interaction found ($F(6,132) = 1.886$, N.S.) (see Fig.8.1).

Analysis of choice behaviour by delay indicated no main effect of test day ($F(6,132) = 0.921$, N.S.) and furthermore demonstrated that animal's choice was highly sensitive to delay ($F(4,88) = 107.441$, $p < 0.001$). Post hoc analysis revealed that selection of the larger delayed reward

decreased significantly with increasing delays (all $p < 0.001$), with choice across all delays differing significantly from other delay trials. Further supporting the stability of choice across delay, was the non-significant day \times delay interaction shown ($F(24, 528) = 0.569$, N.S.). Analysis revealed no group differences in choice behaviour across delay conditions during BL. This was supported statistically by the non significant between group effect and group \times delay interaction found ($F(1,22) = 0.118$, N.S.; $F(4,88) = 0.192$, N.S., respectively). Furthermore, analysis indicated a lack of significant group \times day and group \times delay \times day interaction on choice data ($F(6,132) = 1.647$, N.S.; $F(24, 528) = 1.256$, N.S., respectively) (see also Fig. 8.2(a-e)).

8.2.2.1.2. Speed of Responding

Table 8.1 summarises speed of responding measures in the delayed reinforcement task during baseline. Prior to chronic drug treatment latency to initiate trials was both stable ($F(6,132) = 1.337$, N.S.) and comparable across treatment groups ($F(1,22) = 0.334$, N.S.). Furthermore, the speed at which the delayed and immediate reward were chosen and the latency at which the reward was collected was also stable across baseline ($df = 1, 22$; all $F \geq 0.481$, N.S.) and did not differ between groups ($df = 1, 22$; all $F \geq 0.268$, N.S.). No significant group \times delay interactions were shown across analyses of speed of responding measures during this stage ($df = 6,132$; all $F \geq 0.268$, N.S.).

8.2.2.2. Chronic Administration of Nicotine

8.2.2.2.1. Choice Behaviour

Chronic nicotine treatment resulted in a decrease in overall choice of delayed reward from BL. As shown in Fig. 8.1 nicotine treated animals demonstrated a lower percentage choice of delayed reward in comparison to saline treated animals that was supported statistically by the significant main effect of group ($F(1,21) = 6.267$, $p = 0.021$). Group differences however were not found to interact with test day ($F(5, 105) = 0.393$, N.S.). Although not supported statistically, as shown in Fig. 8.1, the decrease in choice of delayed reward was at its greatest at the early stages of drug treatment following which a gradual return to baseline levels of choice was observed.

Choice behaviour across delays during individual test sessions is shown in Fig. 8.2 (a-e). A clear trend was shown in nicotine animals for choice of the larger reward across 10, 20, 40 and 60 second delays to decrease in relation to average BL choice, whilst choice performed by saline animals remained relatively unchanged. These trends however failed to reach significance with a lack of between group effect and group \times day interactions found across analyses of all delays ($df = 1, 21$; all $F \geq 2.211$, N.S.; $df = 5, 105$; all $F \geq 0.393$, N.S., respectively). Changes in patterns of choice however were not displayed in the zero delay condition, with animals across

treatment groups continuing to select the larger reward to the greatest extent at this delay. Analysis of choice behaviour at this delay revealed no significant main effect of group or group x test day interaction ($F(1,21) = 0.175$, N.S.; $F(3.498, 73.458) = 0.497$, N.S.).

8.2.2.2.2. Speed of Responding

Table 8.2 summaries the speed of responding during the chronic administration of drug treatment. Despite a demonstrated trend for nicotine treated animals to more rapidly initiate trials in comparison to baseline performance, no significant treatment group differences were however found, with an absence of both significant between group ($F(1,21) = 1.016$, N.S.) and group x day interaction shown ($F(1.825, 38.329) = 1.236$, N.S.). Treatment groups furthermore were found not to differ on the speed at which animal's lever responded when selecting the delivery of either the immediate or delayed reward (all $F(1,21) \leq 2.113$, N.S.). Group differences on response latencies in addition were found not to interact with test day (all $F(5,105) \leq 1.236$, N.S.).

Analysis of the speed at which animals collected reward following both an immediate and delayed reward choice demonstrated no significant main effect of treatment group ($F(1,22) = 0.099$, N.S.; $F(1,21) = 0.511$, N.S., respectively). Furthermore, no significant group x test day interaction were displayed on magazine latency following an immediate choice ($F(5,110) = 1.079$, N.S.), or delayed reward choice ($F(5, 105) = 1.757$, N.S.) (see Table 8.2).

8.2.2.3. Nicotine Withdrawal Week One

8.2.2.3.1. Choice Behaviour

Following the initiation of spontaneous withdrawal no significant differences were observed on overall choice of delayed reward during the first week of drug withdrawal. No significant between group or group x test day interaction were found ($F(1,21) = 2.584$, N.S.; $F(3.846, 80.756) = 1.120$, N.S.). As shown in Fig. 8.1, the percentage choice of delayed reward of animals experiencing nicotine withdrawal was comparable to that of saline treated animals up until 84 hours post termination of treatment. Following this time point however a clear trend was shown for the preference of delayed reward to decrease in nicotine treated animals.

Choice of reward by delay demonstrated no significant differences across delays between treatment groups, with a lack of both group main effects and group x test day interactions shown ($F(1,21) \leq 3.142$, N.S.; $F(36,126) \leq 3.117$, N.S., respectively). During early onset of withdrawal, at 10, 20 and 40 seconds delay, the reduction in choice of larger reward that was evident during chronic nicotine treatment, had returned to levels similar to that of saline treated animals and BL (Fig. 8.2 (a-e)). Evident also from Fig. 8.2 (a-e) was a trend shown for saline treated animals to decrease discounting of delayed reward in comparison to BL choice

behaviour.

8.2.2.3.2. Speed of Responding

Latency to initiate trials during withdrawal week one did not differ between treatment groups, with analysis demonstrating a non-significant main effect of group and group x test day interaction ($F(1,21) = 29.895$, N.S.; $F(4.036, 84.748) = 0.973$, N.S.). ANCOVA of the speed at which rewards were selected revealed that whilst no significant between group difference was observed on immediate reward response latency, nicotine animals responded significantly faster in comparison to saline animals when selecting the delayed reward ($F(1,21) = 1.182$, N.S.; $F(1,21) = 4.720$, $p = 0.041$, respectively). Inspection of means summarised in Table 8.3 demonstrate, however, that this effect was aided by the observed slower latency relative to BL performed by saline treated animals. The speed at which the delayed reward was chosen by animals experiencing nicotine withdrawal varied only minimally from average BL performance. Analysis of both measures of responses revealed no significant group x test day interaction ($F(3.423, 71.890) = 1.814$, N.S.; $F(3.625, 76.128) = 1.462$, N.S., respectively).

No significant differences were demonstrated on latency to collect reward following either an immediate ($F(1,22) = 0.568$, N.S.) or delayed reward choice ($F(1,21) = 0.023$, N.S.). Magazine latency by group, furthermore, was not found to interact with test day, for either the collection of immediate or delayed reward ($F(6,132) = 0.725$, N.S.; $F(3.348, 70.317) = 1.850$, N.S., respectively) (see Table 8.3).

Table 8.1: Speed of responding in the delayed reinforcement task during baseline week.

Behavioural Measure		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Initiation latency	N	1.19 ± 0.16	1.21 ± 0.17	1.15 ± 0.16	1.13 ± 0.15	1.05 ± 0.11	1.13 ± 0.13	1.03 ± 0.12
	S	1.04 ± 0.07	1.08 ± 0.11	1.00 ± 0.09	0.98 ± 0.09	1.00 ± 0.08	1.00 ± 0.11	0.99 ± 0.08
Immediate response latency	N	0.69 ± 0.05	0.71 ± 0.07	0.63 ± 0.04	0.65 ± 0.04	0.71 ± 0.05	0.64 ± 0.04	0.72 ± 0.05
	S	0.81 ± 0.08	0.78 ± 0.06	0.77 ± 0.07	0.80 ± 0.07	0.76 ± 0.04	0.70 ± 0.04	0.73 ± 0.06
Delayed response latency	N	0.74 ± 0.05	0.74 ± 0.05	0.75 ± 0.05	0.75 ± 0.05	0.80 ± 0.06	0.80 ± 0.07	0.78 ± 0.06
	S	0.81 ± 0.06	0.81 ± 0.06	0.85 ± 0.08	0.80 ± 0.07	0.81 ± 0.07	0.81 ± 0.08	0.84 ± 0.08
Immediate magazine latency	N	0.23 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.21 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.23 ± 0.03
	S	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.03	0.22 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.02
Delayed magazine latency	N	1.32 ± 0.23	1.73 ± 0.70	1.60 ± 0.46	1.48 ± 0.33	1.63 ± 0.43	1.48 ± 0.32	1.46 ± 0.30
	S	1.90 ± 0.35	2.19 ± 0.33	2.42 ± 0.34	2.15 ± 0.35	2.05 ± 0.38	2.04 ± 0.26	2.03 ± 0.35

Table 8.1: Each value represents the mean latency (seconds) ± SEM.

Table 8.2: Speed of responding in the delayed reinforcement task during chronic administration of nicotine.

Behavioural Measure		Average BL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Initiation latency	N	1.13 ± 0.14	0.93 ± 0.11	1.01 ± 0.13	0.97 ± 0.09	1.08 ± 0.12	1.07 ± 0.12	1.10 ± 0.10
	S	1.01 ± 0.08	0.98 ± 0.09	0.97 ± 0.10	0.96 ± 0.10	0.98 ± 0.07	1.00 ± 0.10	1.03 ± 0.10
Immediate response latency	N	0.68 ± 0.04	0.77 ± 0.06	0.79 ± 0.05	0.78 ± 0.05	0.70 ± 0.04	0.76 ± 0.06	0.70 ± 0.04
	S	0.76 ± 0.05	0.78 ± 0.06	0.75 ± 0.06	0.75 ± 0.06	0.77 ± 0.08	0.76 ± 0.07	0.76 ± 0.07
Delayed response latency	N	0.77 ± 0.05	0.74 ± 0.04	0.78 ± 0.04	0.84 ± 0.05	0.80 ± 0.05	0.77 ± 0.05	0.76 ± 0.08
	S	0.82 ± 0.07	0.83 ± 0.07	0.87 ± 0.07	0.84 ± 0.07	0.83 ± 0.07	0.89 ± 0.07	0.90 ± 0.08
Immediate magazine latency	N	0.23 ± 0.03	0.23 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
	S	0.20 ± 0.02	0.21 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.22 ± 0.03
Delayed magazine latency	N	1.52 ± 0.36	1.23 ± 0.28	1.14 ± 0.24	0.86 ± 0.19	1.27 ± 0.28	1.13 ± 0.26	1.27 ± 0.41
	S	2.11 ± 0.24	1.57 ± 0.36	2.14 ± 0.54	2.15 ± 0.46	2.21 ± 0.28	2.09 ± 0.55	1.51 ± 0.25

Table 8.2: Each value represents the mean latency (seconds) ± SEM.

Table 8.3: Speed of responding in the delayed reinforcement task during nicotine withdrawal week one.

Behavioural Measure		Average BL	12 hours	36 hours	60 hours	84 hours	108 hours	132 hours	156 hours
Initiation latency	N	1.13 ± 0.14	1.30 ± 0.13	1.13 ± 0.14	1.16 ± 0.11	1.19 ± 0.16	1.15 ± 0.15	1.19 ± 0.13	1.06 ± 0.10
	S	1.01 ± 0.08	1.05 ± 0.13	1.06 ± 0.13	0.94 ± 0.10	1.11 ± 0.12	0.92 ± 0.10	1.06 ± 0.14	0.97 ± 0.08
Immediate response latency	N	0.68 ± 0.04	0.68 ± 0.04	0.66 ± 0.05	0.65 ± 0.05	0.66 ± 0.05	0.69 ± 0.04	0.64 ± 0.04	0.69 ± 0.06
	S	0.76 ± 0.05	0.86 ± 0.08	0.83 ± 0.07	0.79 ± 0.10	0.86 ± 0.06	0.71 ± 0.07	0.75 ± 0.07	0.76 ± 0.08
Delayed response latency	N	0.77 ± 0.05	0.81 ± 0.08	0.74 ± 0.05	0.72 ± 0.05	0.76 ± 0.06	0.74 ± 0.06	0.74 ± 0.06	0.74 ± 0.06
	S	0.82 ± 0.07	0.94 ± 0.10	0.86 ± 0.08	0.87 ± 0.08	0.86 ± 0.09	0.86 ± 0.09	0.93 ± 0.12	0.92 ± 0.09
Immediate magazine latency	N	0.23 ± 0.03	0.22 ± 0.03	0.22 ± 0.02	0.23 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.22 ± 0.03	0.21 ± 0.02
	S	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.18 ± 0.01	0.20 ± 0.02	0.23 ± 0.03
Delayed magazine latency	N	1.52 ± 0.36	1.96 ± 0.46	1.73 ± 0.52	1.35 ± 0.30	1.22 ± 0.25	1.20 ± 0.28	1.10 ± 0.25	1.31 ± 0.32
	S	2.11 ± 0.24	1.76 ± 0.36	1.84 ± 0.39	1.90 ± 0.40	2.42 ± 0.48	2.21 ± 0.41	2.40 ± 0.43	2.05 ± 0.63

Table 8.3: Each value represents the mean latency (seconds) ± SEM.

8.2.2.3.3 Somatic Withdrawal Signs

Animals experiencing nicotine withdrawal displayed a rapid sharp elevation in the total frequency of somatic signs beginning at 6 hours post termination of treatment, reaching peak frequency at 18 hours of nicotine withdrawal (see Fig.8.3). Analysis demonstrated a significant main effect of both time and treatment group ($F(12, 264) = 13.973$, $p < 0.001$; $F(1, 22) = 15.967$, $p < 0.001$, respectively) in addition to a group \times time interaction ($F(12, 264) = 5.845$, $p < 0.001$). Further analysis revealed whilst no significant differences between treatment groups were displayed during BL and chronic drug treatment (all $t \leq 1.065$, $df = 22$, N.S.), animals in nicotine withdrawal however exhibited a significantly greater frequency of somatic signs from 6 hours until 109.5 hours post termination of chronic drug treatment (all $t \geq 3.400$, $df = 22$, $p \leq 0.003$).

Examination by treatment group displayed no evidence of an increase in frequency of overall somatic signs in saline treated animals, with a lack of significant main effect of test session found ($F(5.962, 56.993) = 2.2215$, N.S). This was in contrast to the highly significant effect shown in the nicotine treated animals, supporting further the presence of a somatic nicotine withdrawal syndrome in this treatment group ($F(5.333, 63.992) = 20.155$, $p < 0.001$). Post hoc analysis revealed significantly elevated somatic signs at 6, 13.5, 18, 24, 37.5, 61.5, 85.5, 133.5 hours post pump removal in comparison to BL frequency (all $p < 0.01$).

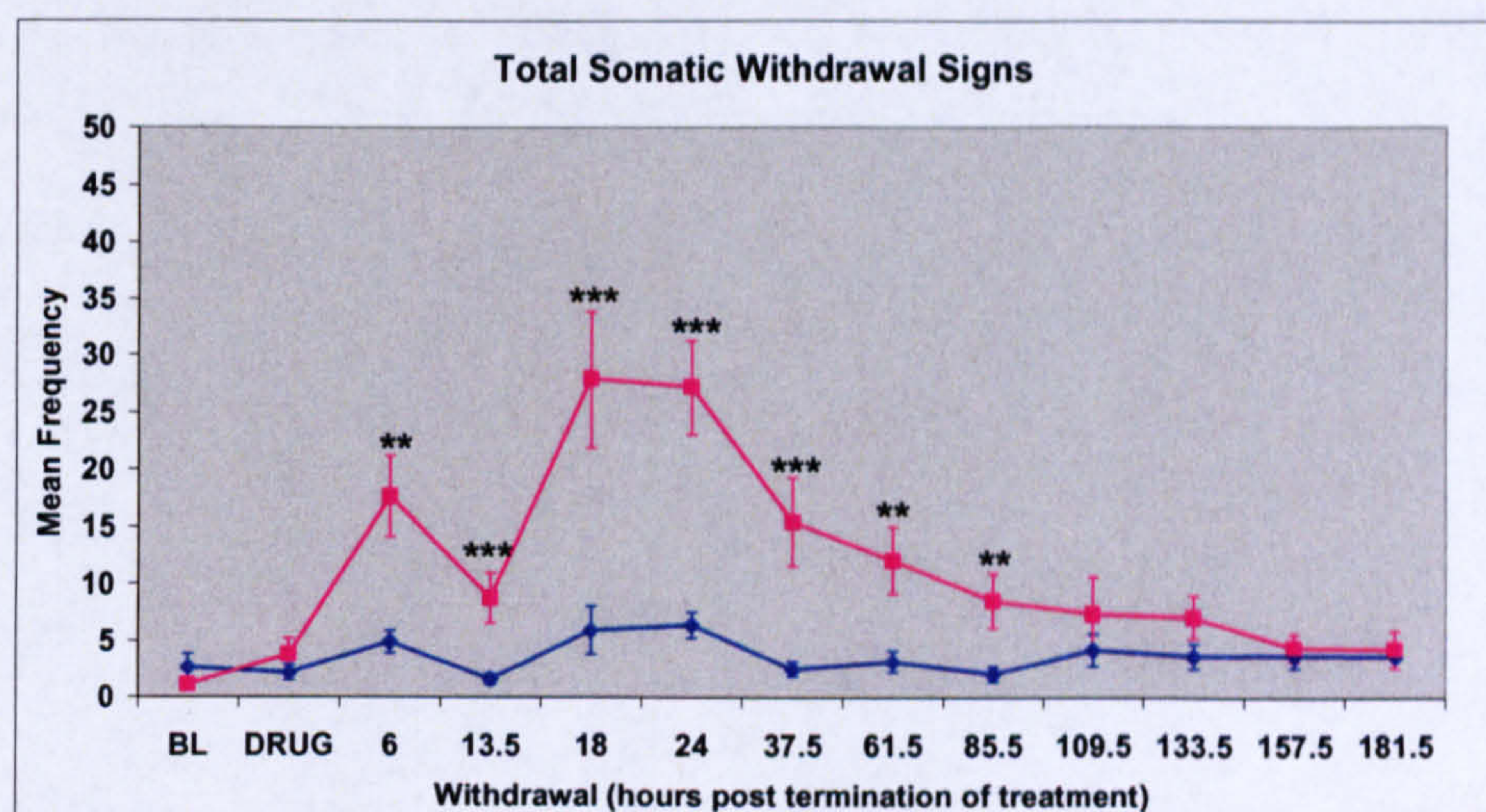


Fig. 8.3: Total Somatic Withdrawal Signs: the frequency of overall somatic signs displayed during spontaneous withdrawal. Each point represents the mean number of trials \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ indicate significant differences between nicotine and saline treatment groups.

Table 8.4 displays the frequency of individual categories of somatic signs observed during spontaneous withdrawal. Animals experiencing nicotine withdrawal exhibited more frequently gasps and writhes in comparison to saline treated from as early as 6 hours post pump removal and continued for a time course of 84.5 hours (all $U \geq 22.00$, $N = 24$, $p \leq 0.50$). Teeth chattering and chews were furthermore a more commonly observed symptom in animals in nicotine in

withdrawal, reaching significance at 6, 18, 24, 37.5, 61.5, 85.5, 133.5 hours post termination of treatment in comparison to saline treated animals (all $U \geq 7.00$, $N = 24$, $p \leq 0.049$). Shakes and tremors were only significantly elevated in previously treated nicotine animals at 18 and 24 hours of withdrawal (all $U \geq 28.00$, $N = 24$, $p \leq 0.01$). Finally, the most commonly exhibited symptoms in animals experiencing nicotine withdrawal were those of scratches and footlicks. Both somatic signs, in cooperation in the miscellaneous observational category, reached significance in comparison to saline treated animals at 6, 18, 24, 37.5, 61.5 post termination of treatment (all $U \geq 20.50$, $N = 24$, $p \leq 0.020$).

8.2.2.4. Nicotine Withdrawal Week Two

8.2.2.4.1. Choice Behaviour

During the second week of withdrawal nicotine treated animals consistently chose the delayed larger reward to a lesser extent than saline treated animals ($F(1,21) = 6.001$, $p = 0.023$). Group differences in choice behaviour were not found to interact with test day ($F(3.554, 74.627) = 0.829$, N.S.) (see Fig. 8.1).

The effect of nicotine withdrawal on choice behaviour was furthermore delay dependent. Analysis of choice behaviour across delays demonstrated a significantly lower choice of the larger delayed reward made by animals experiencing nicotine withdrawal at the 40 second delay condition ($F(1,21) = 4.160$, $p = 0.05$). As shown in Figs. 8.2 (d), the significant difference observed between treatment groups was aided by the coincided slight increase in choice of delayed reward by the saline treatment group. No significant group x test day interaction was observed at this delay ($F(3.752, 78.799) = 0.645$, $p = 0.694$). Despite the additional observed lower choice of delayed reward across 10, 20, 60 delay conditions in animals experiencing nicotine withdrawal (see Fig. 8.2 (a-e)), differences in choice across all other delay trial blocks failed to reach significance, as indicated by the non-significant between group main effects and group x test day interactions shown (all $F(1,21) \leq 3.506$, N.S.; $F(36,126) \leq 1.409$, N.S., respectively).

Table 8.4 Somatic signs of spontaneous withdrawal during withdrawal week one.

Behavioural Measure		Hours Post Termination of Treatment													
		BL	Drug	6 hours	13.5 hours	18 hours	24 hours	37.5 hours	61.5 hours	85.5 hours	109.5 hours	133.5 hours	157.5 hours	181.5 hours	
Gasps and writhes	Nicotine	0.00	0.00	0.69*	1.77*	1.31*	1.85**	0.85*	0.85*	0.69*	0.31	0.15	0.15	0.15	
		±0.00	±0.00	±0.29	±0.60	±0.49	±0.63	±0.30	±0.36	±0.35	±0.17	±0.10	±0.10	±0.15	
	Saline	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	
		±0.00	±0.00	±0.00	±0.18	±0.00	±0.00	±0.00	±0.00	±0.00	±0.12	±0.00	±0.00	±0.00	
Teeth chatters and chews	Nicotine	0.00	0.00	3.92***	1.61	3.53***	5.15**	1.84**	1.38**	0.92*	0.23	0.77*	0.23	0.00	
		±0.00	±0.00	±0.74	±0.63	±0.65	±1.48	±0.52	±0.38	±0.29	±0.23	±0.34	±0.23	±0.00	
	Saline	0.18	0.09	0.09	0.27	0.18	0.36	0.18	0.00	0.09	0.18	0.00	0.09	0.00	
		±0.12	±0.09	±0.09	±0.14	±0.12	±0.27	±0.12	±0.00	±0.09	±0.18	±0.00	±0.09	±0.00	
Shakes and Tremors	Nicotine	0.00	0.46	2.15	0.69	1.92**	2.15*	0.92	0.54	0.38	0.38	0.15	0.15	0.07	
		±0.00	±0.31	±0.47	±0.29	±0.47	±0.46	±0.29	±0.33	±0.21	±0.18	±0.10	±0.10	±0.07	
	Saline	0.09	0.36	1.09	0.09	0.73	0.82	0.27	0.27	0.27	0.09	0.00	0.45	0.18	
		±0.09	±0.15	±0.25	±0.09	±0.47	±0.44	±0.14	±0.19	±0.14	±0.09	±0.00	±0.21	±0.12	
Miscellaneous	Nicotine	1.15	3.38	10.92*	4.69	21.15**	18.08**	11.85*	9.31*	6.54	6.46	6.00	3.92	4.07	
		±1.22	±4.39	±4.77	±6.25	±6.16	±8.87	±5.47	±5.50	±5.50	±3.04	±2.51	±5.56	±2.83	
	Saline	2.36	1.72	3.73	1.09	6.81	6.63	2.09	2.91	1.73	3.73	3.64	2.91	3.45	
		±1.23	±0.59	±0.81	±0.28	±2.67	±1.47	±0.59	±0.96	±0.69	±1.31	±1.08	±1.03	±0.94	

Table 8.4: Somatic Withdrawal Signs: the frequency of somatic signs displayed during spontaneous withdrawal. Each value represents the mean frequency (seconds) ± SEM. *, p<0.05, **, p<0.01, ***, p<0.001 indicate significant differences between nicotine and saline treatment groups.

8.2.2.4.2 Speed of Responding

Table 8.5 summarises speed of responding measures across groups during withdrawal week two. No significant main effects of treatment group were displayed on the speed at which trials were initiated or latency at which immediate or delayed reward choices were made ($F(1,21) \leq 1.915$, N.S.). Furthermore, across measures of initiation latency and immediate and delayed response latency no significant group \times test interactions were displayed ($F(3.492, 73.228) = 1.131$, N.S.; $F(3.890, 81.6830) = 0.822$, N.S.; $F(3.211, 67.434) = 0.135$, N.S., respectively).

Latency to collect both immediate and delayed rewards was similar across treatment groups with a lack of both significant main effects of group ($F(1,22) = 0.499$, N.S.; $F(1,21) = 0.001$, N.S., respectively) and group \times test interactions shown on both measures ($F(6, 132) = 0.702$, N.S.; $F(6, 126) = 0.966$, N.S., respectively).

8.2.2.5. Nicotine Withdrawal Week Three

8.2.2.5.1. Choice Behaviour

By the third week post termination of treatment, choice behaviour of animals treated chronically with nicotine had returned to BL levels and was similar to the overall choice of the delayed larger reward displayed by the saline treatment group (see Fig. 8.1). Analysis revealed no significant between group or group \times test day interaction on overall choice of delayed reward ($F(1,21) = 0.599$, N.S.; $F(6,126) = 0.616$, N.S., respectively).

Similar choice behaviour across treatment groups was also evident in the choice of delayed reward across delays (see Figs. 8.2 (a-d)). An absence of both main effects of group and group \times test day interactions were shown across all delay conditions within the task ($F(1,21) \leq 1.606$, N.S.; $F(36,126) \leq 1.689$, N.S., respectively). As clearly illustrated in both Figs 8.2 (a-e) across delay conditions nicotine treated animal's choice of the larger reward had returned to levels parallel to that observed prior to the initiation of chronic drug treatment.

8.2.2.5.2. Speed of Responding

Speed of responding within the delayed reinforcement task, tabulated in Table 8.6, was similar across treatment groups during the third week post termination of treatment. No significant main effects of group ($F(1,21) \leq 0.991$, N.S.) or group \times test day interactions ($F(6,126) \leq 0.933$, N.S.) on the speed at which animals initiated trials and selected immediate and delayed rewards were found. Immediate and delayed reward magazine latencies were in addition found not differ across treatment groups, with an absence of both significant between group ($F(1,22) = 3.915$, N.S.; $F(1,21) = 3.151$, N.S., respectively) and group \times test day interactions shown ($F(6,132) = 2.028$, N.S.; $F(6,126) = 1.276$, N.S., respectively).

Table 8.5: Speed of responding in the delayed reinforcement task during nicotine withdrawal week two.

Behavioural Measure		Average BL	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Initiation latency	N	1.13 ± 0.14	1.24 ± 0.20	1.14 ± 0.17	1.06 ± 0.11	0.97 ± 0.10	1.07 ± 0.07	1.07 ± 0.10	1.08 ± 0.11
	S	0.95 ± 0.08	0.96 ± 0.07	0.95 ± 0.06	0.96 ± 0.08	0.97 ± 0.11	0.97 ± 0.08	0.97 ± 0.08	1.04 ± 0.08
Immediate response latency	N	0.68 ± 0.04	0.69 ± 0.04	0.69 ± 0.05	0.71 ± 0.05	0.78 ± 0.08	0.73 ± 0.05	0.70 ± 0.04	0.67 ± 0.03
	S	0.76 ± 0.05	0.77 ± 0.10	0.84 ± 0.11	0.77 ± 0.06	0.74 ± 0.07	0.74 ± 0.05	0.70 ± 0.04	0.74 ± 0.07
Delayed response latency	N	0.77 ± 0.05	0.74 ± 0.05	0.78 ± 0.06	0.78 ± 0.05	0.75 ± 0.06	0.79 ± 0.07	0.80 ± 0.07	0.80 ± 0.07
	S	0.82 ± 0.07	0.85 ± 0.07	0.91 ± 0.08	0.89 ± 0.07	0.84 ± 0.06	0.88 ± 0.07	0.89 ± 0.08	0.87 ± 0.07
Immediate magazine latency	N	0.23 ± 0.03	0.24 ± 0.03	0.22 ± 0.03	0.21 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.22 ± 0.03
	S	0.20 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.21 ± 0.03	0.21 ± 0.02	0.21 ± 0.02	0.24 ± 0.03
Delayed magazine latency	N	1.52 ± 0.36	1.20 ± 0.24	1.43 ± 0.42	1.27 ± 0.27	1.23 ± 0.34	1.28 ± 0.40	1.34 ± 0.34	1.31 ± 0.35
	S	2.11 ± 0.24	1.88 ± 0.58	1.92 ± 0.36	1.57 ± 0.33	1.99 ± 0.25	1.87 ± 0.32	1.74 ± 0.38	1.83 ± 0.33

Table 8.5: Each value represents the mean latency (seconds) ± SEM.

Table 8.6: Speed of responding in the delayed reinforcement task during nicotine withdrawal week three.

Behavioural Measure		Average BL	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Initiation latency	N	1.13 ± 0.14	1.12 ± 0.12	1.15 ± 0.11	1.08 ± 0.11	1.04 ± 0.08	0.97 ± 0.07	1.03 ± 0.10	1.01 ± 0.10
	S	0.95 ± 0.08	1.02 ± 0.07	1.12 ± 0.09	0.96 ± 0.06	0.99 ± 0.08	0.91 ± 0.10	0.86 ± 0.06	0.86 ± 0.09
Immediate response latency	N	0.68 ± 0.04	0.63 ± 0.03	0.69 ± 0.04	0.70 ± 0.05	0.71 ± 0.06	0.73 ± 0.06	0.67 ± 0.03	0.70 ± 0.04
	S	0.76 ± 0.05	0.76 ± 0.07	0.70 ± 0.05	0.74 ± 0.06	0.74 ± 0.06	0.78 ± 0.07	0.74 ± 0.06	0.74 ± 0.06
Delayed response latency	N	0.77 ± 0.05	0.80 ± 0.07	0.78 ± 0.07	0.79 ± 0.07	0.85 ± 0.08	0.80 ± 0.07	0.86 ± 0.08	0.84 ± 0.07
	S	0.82 ± 0.07	0.88 ± 0.09	0.82 ± 0.06	0.89 ± 0.08	0.88 ± 0.06	0.91 ± 0.07	0.94 ± 0.07	0.95 ± 0.07
Immediate magazine latency	N	0.23 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.21 ± 0.03	0.20 ± 0.03	0.20 ± 0.02	0.19 ± 0.03
	S	0.20 ± 0.02	0.21 ± 0.02	0.22 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.03
Delayed magazine latency	N	1.52 ± 0.36	1.15 ± 0.31	1.37 ± 0.29	1.34 ± 0.27	1.05 ± 0.25	0.95 ± 0.20	1.57 ± 0.47	1.15 ± 0.19
	S	2.11 ± 0.24	1.81 ± 0.39	1.78 ± 0.36	1.57 ± 0.38	1.43 ± 0.22	1.63 ± 0.45	1.62 ± 0.50	1.87 ± 0.35

Table 8.6: Each value represents the mean latency (seconds) ± SEM.

8.2.2.6. High vs. Low “Trait” Impulsivity

The rate of exponential decay was determined for each animal during baseline week. This analysis identified a group of highly impulsive animals ($N = 9$) whose rate of discounting of the delayed reward, as represented by k , was significantly greater than the remaining group ($N = 15$) ($t = 5.255$, $df = 22$, $p < 0.001$). A mean k value of 0.5420 ± 0.07 was represented by the high impulsive animals in comparison to a mean k value of 0.2130 ± 0.03 displayed by the low impulsive group. Individual animal k values in addition to R^2 values are summarised in Table 3.1. Appendix 3. Indication that the exponential function provided a good fit of the data, was demonstrated by the average R^2 values of 0.87 ± 0.03 and 0.82 ± 0.04 for the choice data for high and low impulsive animals respectively.

Fig. 8.4 illustrates the average discounting of delayed reward performed by both the high and low impulsive groups during baseline BL. To assess differences in response of high and low impulsive animals to chronic nicotine and withdrawal on choice behaviour, analysis was conducted independently for each of the groups. Across both saline and nicotine treatment groups, $N=6$, and $N= 9$, low impulsive animals were identified respectively. In terms of high impulsive animals, $N= 4$ were present in the nicotine group compared to $N=5$ in the saline treatment group. Fig. 8.5 illustrates and compares overall choice of delayed reward across treatment groups for both high and low impulsive animals. Fig. 8.6 (a-e) illustrate choice by delay.

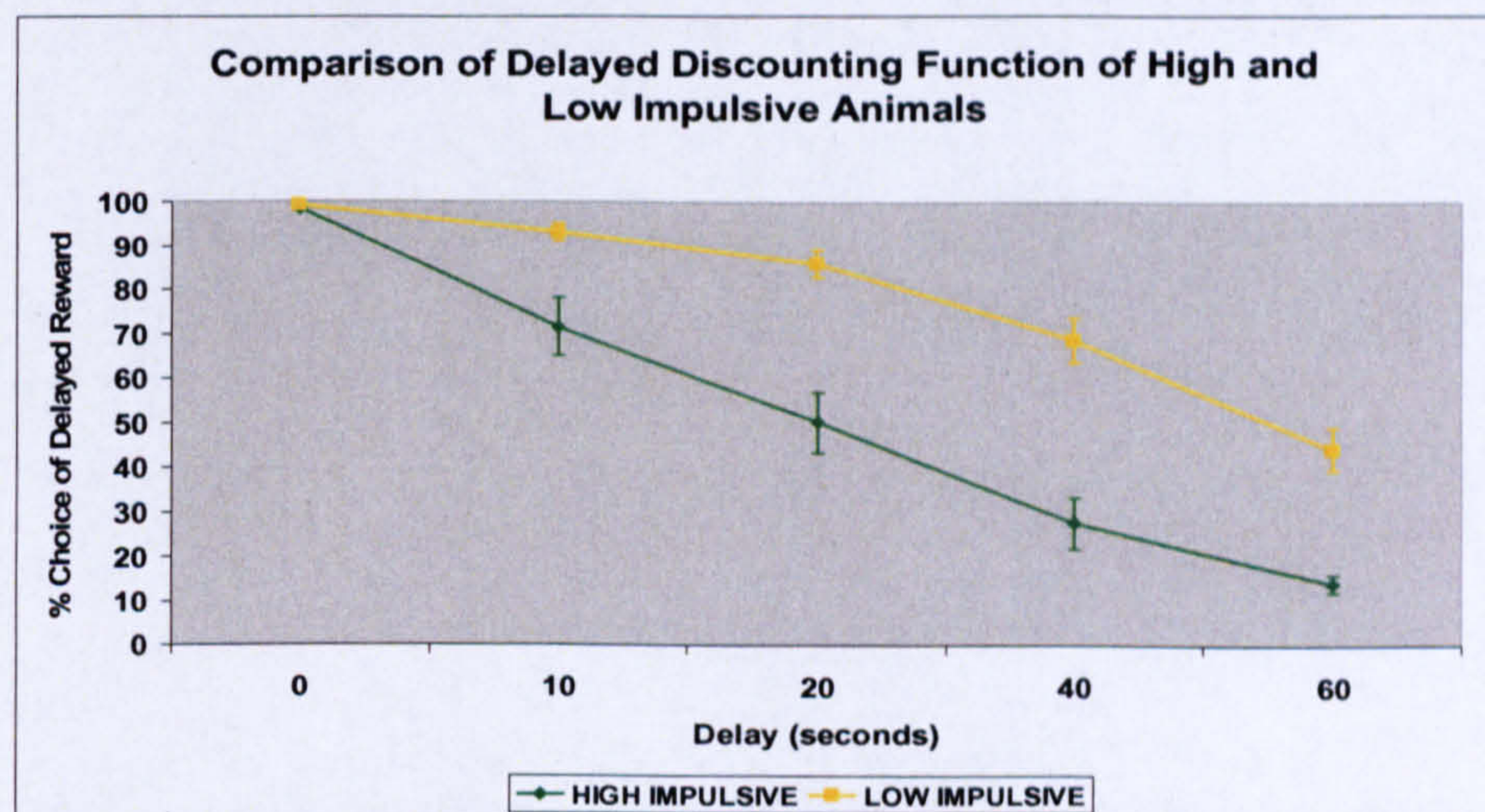


Fig. 8.4. Choice Behaviour: the average rate of discounting of delayed reward during baseline of high and low “trait” impulsive animals. Each point represents the mean percentage choice \pm SEM.

8.2.2.6.1. Baseline

8.2.2.6.1.1. Choice Behaviour

During baseline performance low impulsive animal's overall choice of delayed reward was both stable ($F(4.107, 49.280) = 1.108$, N.S.) and similar across treatment groups, supported statistically by the lack of significant main effect of treatment group ($F(1,12) = 0.276$, N.S.) (see Fig.8.5). Significant, however, was the day x group interaction ($F(4.107, 49.280) = 2.932$, $p = 0.029$). Further analysis however revealed no significant group differences in choice across individual test days (all $t \leq -0.062$, $df = 13$, N.S.). Analysis by treatment group furthermore demonstrated no significant main effect of test day for either saline or nicotine treatment groups ($F(2.433, 7.300) = 0.400$, N.S.; $F(6,42) = 0.577$, N.S., respectively).

Analysis of choice by delay demonstrated no significant main effect of test day ($F(6,78) = 0.089$, N.S.). Choice of delayed reward by low impulsive animals was highly sensitive to delay, supported by the main effect of delay ($F(4,52) = 65.155$, $p < 0.001$). Choice of the larger reward decreased with increasing delays, with choice at all delays differing significantly from the 0 second delay condition (all $p < 0.05$). Further support for the stability of delay dependency in low impulsive animals, was indicated by the non-significant day x delay interaction ($F(24, 312) = 0.505$, N.S.). Delay dependency did not differ across treatment groups with absence of a main effect of group ($F(1,12) = 0.001$, N.S.), group x delay and group x delay x day interactions found ($F(4,52) = 0.077$, N.S.; $F(24, 312) = 1.088$, N.S., respectively) (see Fig. 8.6 (a-e)).

The overall choice of delayed reward displayed by high impulsive animals during BL was also both relatively stable and similar across treatment groups. An exception to this stability was BL day 6 (see Fig. 8.5). During this test session, the saline treatment group displayed a greater percentage choice of the delayed larger reward. Further examination of choice behaviour by delay, also revealed similar levels of preference of the larger reward across treatment groups during all delay conditions (see Fig. 8.6 (a-e)).

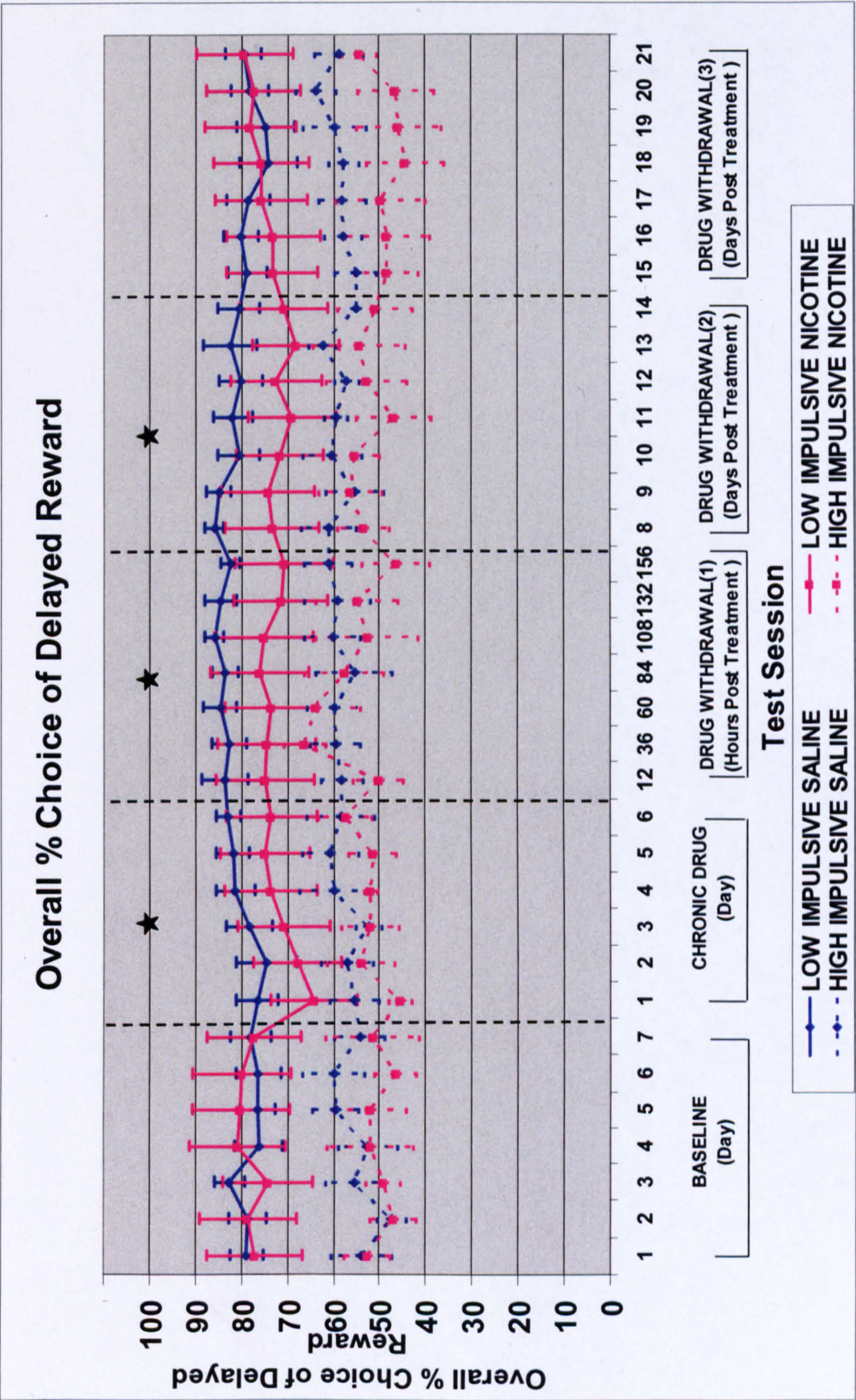


Fig. 8.5. Choice Behaviour: the effects of chronic nicotine administration and withdrawal on overall percentage choice of delayed reward in high and low impulsive animals. Each point represents the mean percentage choice. ★ $p < 0.05$ Main effect of group

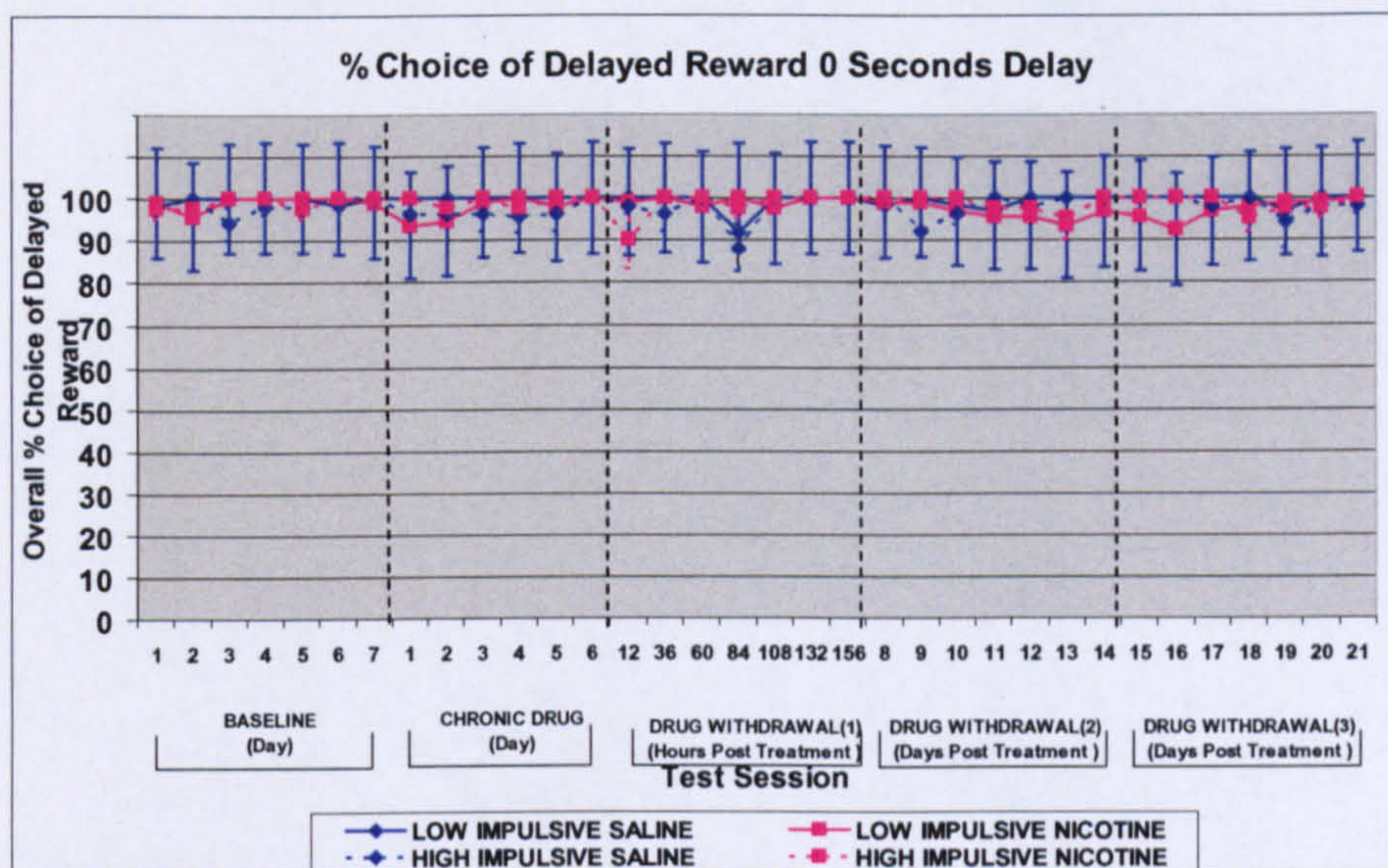


Fig 8.6(a)

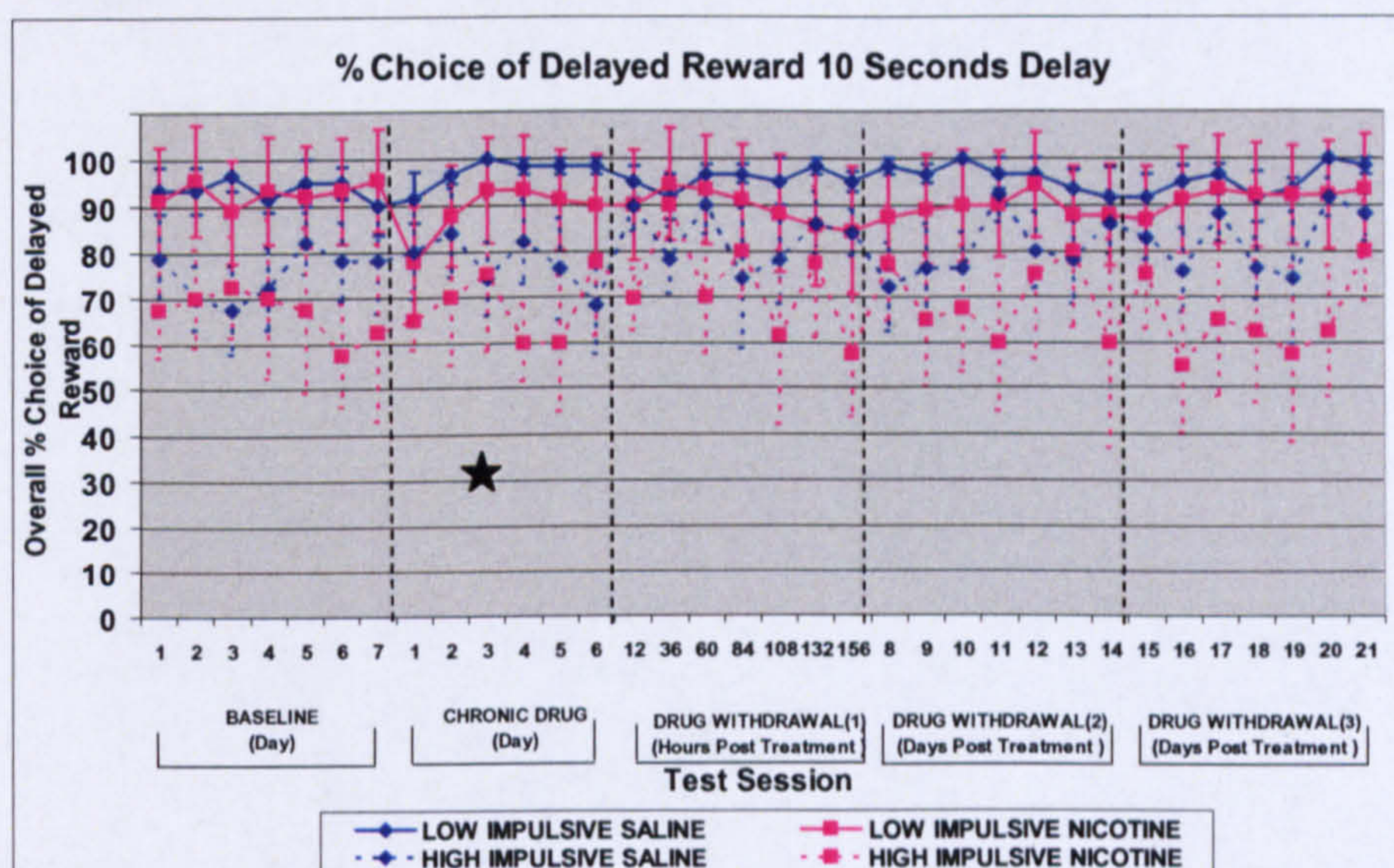


Fig. 8.6(b)

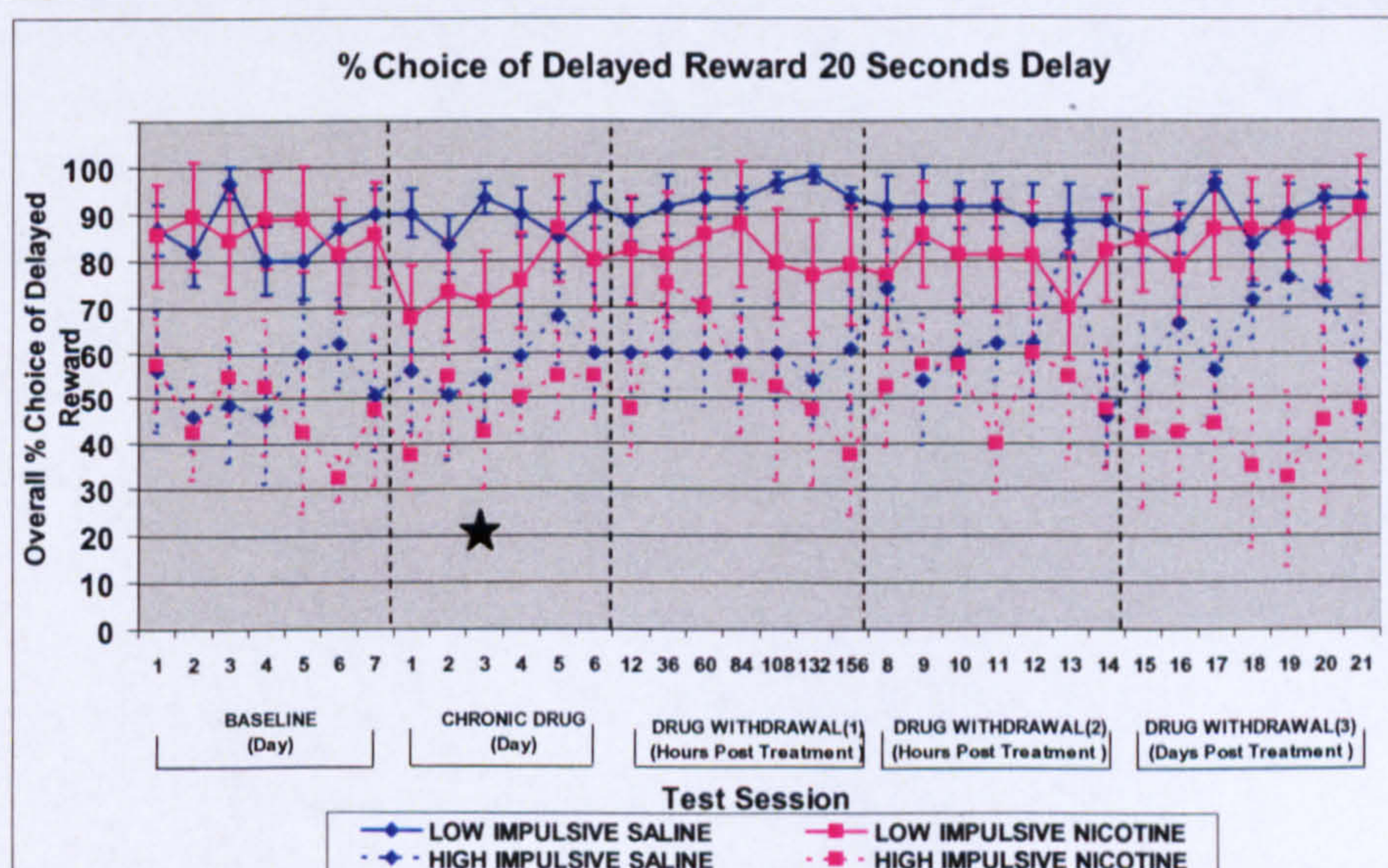


Fig. 8.6(c)

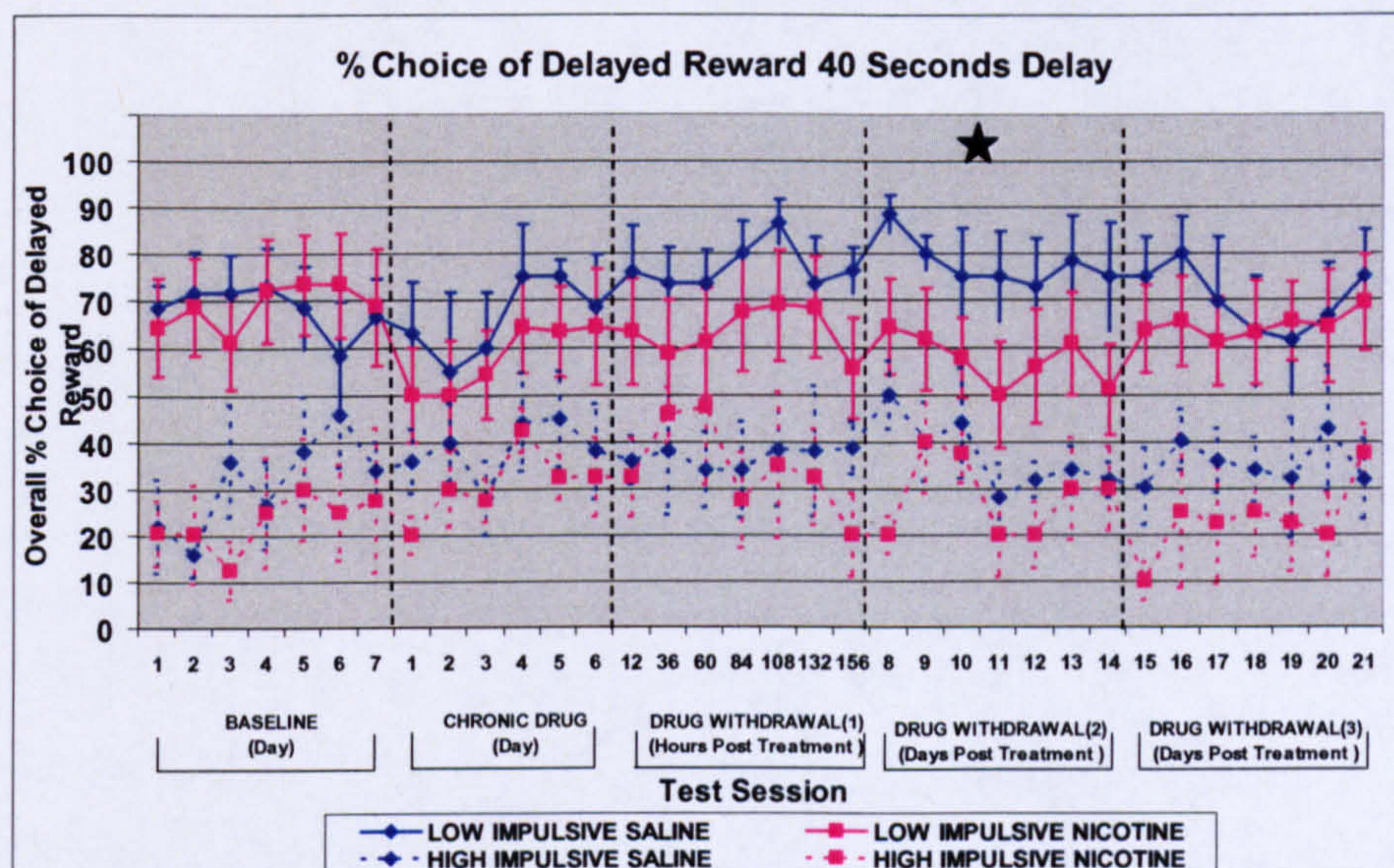


Fig. 8.6(d)

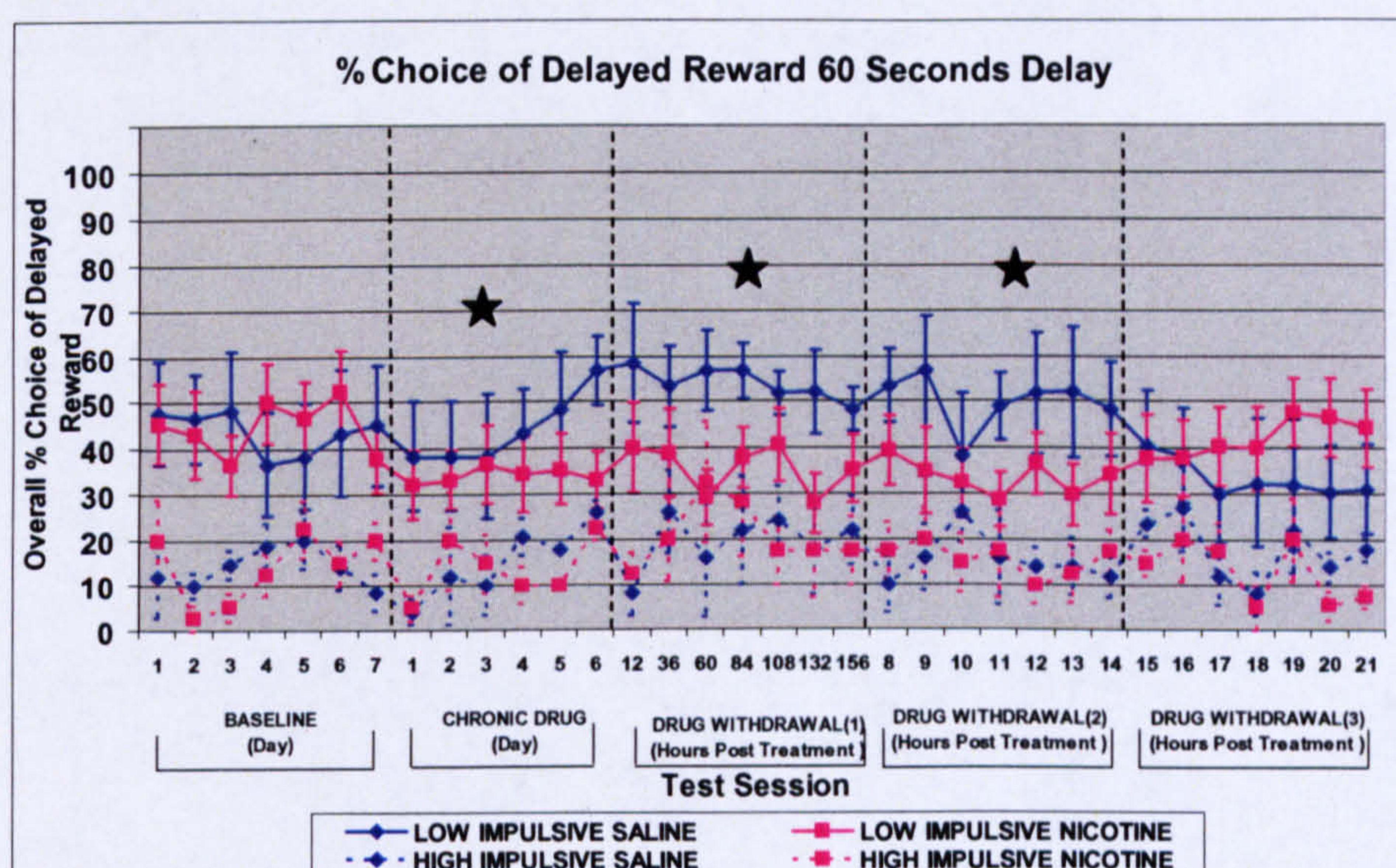


Fig. 8.6(e)

Fig. 8.6 (a-e): Choice Behaviour: the effects of chronic nicotine administration and withdrawal on percentage choice of delayed reward during each delay in high and low impulsive animals. Each point represents the mean percentage choice. ★ $p < 0.05$ Main effect of group

8.2.2.6.2. Chronic Administration of Nicotine

8.2.2.6.2.1. Choice Behaviour

When treated chronically with nicotine low impulsive animals demonstrated a substantial reduction in overall choice of delayed reward in comparison to saline treated animals ($F(1,12) = 10.763$, $p = 0.007$), an effect that was consistent over the treatment period and was at its maximum during the first test session (see Fig.8.5). Overall choice of delayed reward by treatment group was however found not to interact with test day ($F(5,60) = 0.147$, N.S.). The increase in impulsive choice was furthermore delay dependent in nicotine treated animals. As illustrated in Fig. 8.6 (a-e) choice of delayed reward was found to be significantly lower at 10, 20 and 60 second delay conditions in comparison to choice behaviour displayed by the saline control group (all $F(1,12) \geq 5.228$, $p \leq 0.041$). In contrast, choice behaviour during drug

treatment was similar across treatment groups at 0 and 40 second delays (all $F(1,12) \leq 2.599$, N.S.). Analyses indicated no significant interactions between group and test day across all delay conditions (all $F \leq 1.345$; $df \geq 2.169$, 26.943, N.S.) (see Fig. 8.6 (a-e)).

The chronic administration of nicotine in contrast had minimal affect on impulsive choice in high impulsive animals. As clearly illustrated in Fig. 8.5, overall percentage choice of delayed reward in nicotine treated animals failed to change dramatically from BL, with choice behaviour being comparable to that of saline treated animals during drug treatment. Examination of choice of reward by delay further supports the lack of response to chronic nicotine. Across delays discounting of delayed reward in nicotine treated animals remained similar to both BL and choice behaviour displayed by saline treated animals (see also 8.6 (a-e)).

8.2.2.6.3. Nicotine Withdrawal Week One

8.2.2.6.3.1. Choice Behaviour

In comparison to BL, the reduction in overall choice of delayed reward by low impulsive nicotine treated animals continued following the termination of drug treatment (see Fig.8.5). Analysis revealed a significant main effect of treatment group aided by the slight increase in overall percentage choice of delayed reward displayed by the saline treated animals ($F(1,12) = 6.904$, $p = 0.020$). The treatment group effect was found not interact with test day ($F(2,811, 34.566) = 0.393$, N.S.). As demonstrated in Figs. 8.6 a-e, animals experiencing nicotine withdrawal displayed a greater reduction in choice of the delayed larger reward across delays in comparison to control animals, with the exception of 0 second delay trials. In contrast, saline treated animals appeared to increase their choice of the delayed reward at 40 and 60 second delay trials relative to baseline. Analysis of choice by delay however revealed that impulsive choice between treatment groups only differed significantly at 60 seconds delay ($F(1,12) = 6.904$, $p = 0.022$). An absence of significant between group effects were indicated across all remaining delays (all $F(1,21) \leq 2.460$, N.S.). Finally no significant group x test day interactions were found across all delay conditions (all $F \leq 1.817$, $df \geq 2.198$, 26.375, N.S.).

The termination of nicotine treatment in contrast in high impulsive animals led to a decrease in impulsive choice in comparison to baseline and saline control animals, peaking at 36 hours post pump removal (see Fig.8.5). Overall choice of delayed reward remained greater in nicotine treated animals from 36 until 84 hours of drug withdrawal. As illustrated in Fig. 8.6(a-e) this was as a result of the greater choice of delayed reward at 20, 40 and 60 second delay conditions in comparison to both BL and choice displayed by the saline treatment group.

8.2.2.6.4. Nicotine Withdrawal Week Two

8.2.2.6.4.1. Choice Behaviour

Low impulsive animals experiencing the second week of withdrawal continued to display reduced choice of the delayed, larger reward in comparison to the saline treated animals (see Fig. 8.5). Analysis of overall percentage choice of delayed reward revealed a significant main effect of treatment group that did not interact with test day ($F(1,12) = 8.589$, $p = 0.013$; $F(6,72) = 0.440$, N.S., respectively). Choice of delayed reward was significantly reduced at the higher 40 and 60 second delay conditions in comparison to saline control animals (all $F(1,12) \geq 6.202$, $p \leq 0.026$) (see Figs. 8.6 (a-e)). Group differences however were not found to interact with test day (all $F(6,72) \leq 0.510$, N.S.). Similar choice behaviour was displayed across treatment groups during 0, 10 and 20 second delays, indicated by the absence of both significant between group (all $F(1,12) \leq 1.899$, N.S.) and group x test day interactions shown (all $F \leq 0.599$, $df \geq 2.198$, 26.375, , N.S.).

During the second week post termination of nicotine treatment, high impulsive animals displayed no evidence of differences in overall choice of delayed reward in comparison to the saline treatment group (see Fig. 8.5). The lack of difference in choice behaviour was indicated furthermore across delay conditions as illustrated in Fig. 8.6(a-e). Saline treated animals displayed in contrast a slight increase in choice of the delayed, larger reward.

8.2.2.6.5. Nicotine Withdrawal Week Three

8.2.2.6.5.1. Choice Behaviour

By the third week post termination of treatment overall choice of delayed reward by low impulsive nicotine treated animals had returned to BL. A lack of difference between treatment groups on overall percentage choice of delayed reward was displayed, with no significant main effect treatment group or group x test day interaction found ($F(1,12) = 0.095$, N.S.; $F(6,72) = 0.936$, N.S., respectively) (see Fig. 8.5). Analysis of choice across delays demonstrated furthermore comparable reward selection between treatment groups with both main effects of group and group x test day interactions failing to reach significance (all $F(1,12) \leq 2.021$, N.S.; all $F(6,72) \leq 1.212$, N.S., respectively) (see Figs. 8.6 (a-e))

In contrast, high impulsive animals displayed the greatest reduction of delayed reward in comparison to the saline treatment during the third week of withdrawal (Fig. 8.6). Nicotine treated animals displayed a decreased pattern of overall choice of delayed reward in comparison to baseline performance from day 18 to 20 post termination of treatment. During these test sessions was the coincided increase in choice of delayed reward by the saline treatment group, which aided and led to the display of higher levels of impulsive choice in nicotine treated animals until day 21. Examination of choice by delay revealed that this was due to the reduction

in choice of delayed reward by nicotine treated animals during the 10 and 20 second delays, where in contrast an increase in choice was shown by saline treated animals (see Fig. 8.6(a-e)). In comparison to BL, however, nicotine treated animals appeared to differ minimally in choice. Saline treated animals however displayed evidence OF an increase in choice of the delayed reward.

8.3: EXPERIMENT 8 EXAMINATION OF THE EFFECTS OF ACUTE NICOTINE CHALLENGES ON IMPULSIVE CHOICE FOLLOWING PREVIOUS EXPOSURE TO NICOTINE

8.3.1. METHOD

8.3.1.1. Subjects

Subjects were 24 adult male Lister Hooded rats (Charles River, UK). All animals had completed Experiment 7 and had been previously chronically treated with either nicotine (n = 13) or saline (n = 11). Subject details have been previously outlined in methodology of Experiment 7, section 8.2.1.1. At the start of testing, animals weighed approximately 400-440g.

8.3.1.2. Apparatus

Subjects conducted the delayed reinforcement task in two sets of four operant chambers (dimensions 30.5 X 24.1 X 21cm 30.5 X 24.1 X 29.2cm Med Associates Inc., USA). For a more detailed description of all apparatus refer to Chapter 2 section 2.5.1.

8.3.1.3. Behavioural Testing

The behavioural procedure of the delayed reinforcement task has been described previously in the general methodology of Chapter 6, section 6.2.1.3. The behavioural measures assessed in the paradigm are in addition detailed in Chapter 6. Details of subject training have been outlined previously in Experiment 7, section 8.2.1.3.

8.3.1.4. Drugs

Nicotine hydrogen tartrate salt was dissolved in 0.9% saline and the pH adjusted to approximately 6, using 0.1M sodium hydroxide. Nicotine (0, 0.125, 0.25 and 0.5mg/kg) was administered s.c. in a volume of 1ml/kg bodyweight. All doses were calculated as free base and prepared freshly on each test day. Nicotine was administered 10 minutes prior to the operant test session.

8.3.1.4. Design and Procedure

Assessment began three weeks following the initiation of spontaneous withdrawal. All animals had returned to BL level of impulsive choice and no significant differences were observed in comparison to choice behaviour recorded prior to the initiation of chronic drug treatment.

Furthermore, no differences between groups in terms of their choice behaviour were present.

The effects of acute nicotine challenges were assessed using a mixed design, with treatment group (either previously chronically treated with saline or nicotine) as the between subject factor and acute drug treatment condition as the within subject factor. Treatments were administered according to a randomised Latin square design with a minimum of 72 hours separating consecutive drug treatments. It was ensured that animals had returned to BL performance prior to subsequent treatment doses being administered. Baseline was defined as overall choice of the delayed reward deviating no greater than 10% from the level of impulsive choice of delayed reward demonstrated by the subject prior to the initiation of the acute drug challenge regime. During the three days prior to the initiation of acute nicotine challenge, animals were habituated to injection procedures on two occasions, with subjects s.c. injected with 1ml/kg saline 10 minutes prior to the operant session. Experimentation followed standard operant testing procedures, outlined in detail in the general methodology of Chapter 2, section 2.5.2. All operant testing took place during the light phase of their LD cycle between 0830h and 1730h.

Each of the four treatment challenges (0, 0.125, 0.25, and 0.5mg/kg s.c.) were administered 10 minutes prior to the test session following which they were transferred immediately to the operant test room. All injection procedures were conducted in a procedure room separate from both the holding room and operant test laboratory. Comparison of the acute effects of nicotine across treatment groups was conducted over a three week period.

8.3.1.5. Statistical Analysis

To ensure animals had returned to BL levels of performance prior to treatment and no differences were present across treatment groups, choice of delayed reward across delay during the first three days following the third week of withdrawal were compared to the average choice behaviour observed during baseline week prior to chronic drug treatment. Data was then analysed by a three-way mixed measures ANOVA with test session and delay as the within subject factors and treatment group as the between subject factor.

For the examination of differences between treatment groups in response to acute nicotine challenges, results for each behavioural parameter were analysed by a two-way mixed ANOVA, with treatment condition as the between subject factor and treatment dose condition as the within subject factor. Choice of reward by delay, involved a three-way ANOVA being applied to the data, with both treatment condition and delay (0, 10, 20, 40 and 60s) as the within subject variables and treatment group as the between subject factor.

To assess whether BL differences in impulsive choice affected animals response to acute

nicotine on discounting of delayed reward, data from high and low impulsive animals were analysed independently and compared. The examination of acute nicotine challenges on choice behaviour in low impulsive animals was assessed as above. Due to the low sample size of highly impulsive animals, data was not subjected to statistical analysis; descriptive of choice behaviour were instead plotted and explored.

All main effects of analysis were assessed further where appropriate by Bonferroni post hoc comparisons. To examine significant treatment dose x group interactions, one way repeated measures ANOVA examined by group the response across acute drug treatments, whilst a series of independent t-tests compared groups at each treatment dose (Bonferroni correction of $p < 0.0125$). To explore further treatment dose x delay interactions, simple one-way ANOVAs were used to both examine the main effects of treatment across each delay conditions and the main effect of delay at individual treatment doses. Mauchley's test of sphericity was applied to all within subject variables, and when appropriate the degrees of freedom adjusted with the Greenhouse-Geisser correction. The homogeneity of variance of between subject variables was assessed by Levine's test. All data prior to analysis was assessed for normality and transformed where necessary (see also section 2.7). If data could not be successfully transformed, then the non-parametric Friedman test was employed to examine the dose-response to nicotine by treatment group followed where appropriate by Wilcoxon procedures. Mann Whitney U tests were applied to the data to allow comparison of responses by group at each treatment dose. In all cases of analysis α values of $p < 0.05$ were deemed statistically significant.

8.3.2. RESULTS

Failure to initiate trials, make a reward choice and to collect reward following selection, rarely occurred across all treatment doses. Analysis of these parameters was therefore not conducted.

8.3.2.1. Return to Baseline

Comparison of choice behaviour to that of performance prior to the initiation of chronic drug treatment, revealed that choice of delayed reward had returned to BL levels. This was supported by the non-significant main effect of test session ($F(3,66) = 1.780$, N.S.). Choice behaviour was highly sensitive to delay ($F(2.572, 56.592) = 124.612$, $p < 0.001$), with choice of delayed reward being significantly reduced across all delays in comparison to the 0 second delay condition (all $p < 0.001$). Further support that choice behaviour had returned BL was indicated by the non-significant day x delay interaction ($F(12,264) = 1.863$, N.S.). Analysis also revealed that choice behaviour did not differ between treatment groups, as shown by non-significant main effect of group ($F(1, 22) = 0.001$, N.S.). Furthermore support for the similarity of choice at this stage was indicated by the non-significant group x day, group x delay and group x day x delay interactions observed ($F(3,66) = 0.716$, N.S.; $F(4,88) = 1.627$, N.S.; $F(12, 264) = 1.279$, N.S.).

8.3.2.2. Choice Behaviour

Acute administration of nicotine, as shown in Fig. 8.7, significantly decreased preference for the delayed larger reward in a dose related manner ($F(1.972, 43.389) = 21.214, p < 0.001$). Post hoc analysis revealed a significant increase in rate of discounting of delay across all doses tested in comparison to saline control (all $p < 0.001$). Furthermore, the highest 0.5mg/kg dose decreased significantly choice of delayed reward in comparison to the 0.125mg/kg treatment dose. Both treatment groups responded similarly to the acute nicotine challenges with no significant main effect group or group x dose interaction found ($F(1,22) = 0.082, N.S$; $F(1,972, 43.389) = 11.04, N.S.$, respectively). As illustrated in Fig. 8.7 almost identical reductions in overall choice of delayed reward in both treatment groups were observed following 0.25mg/kg and 0.5mg/kg doses of nicotine. Conversely, following the lowest 0.125mg/kg dose, nicotine naïve animals displayed a slight (although not significant) greater sensitivity to the acute challenge.

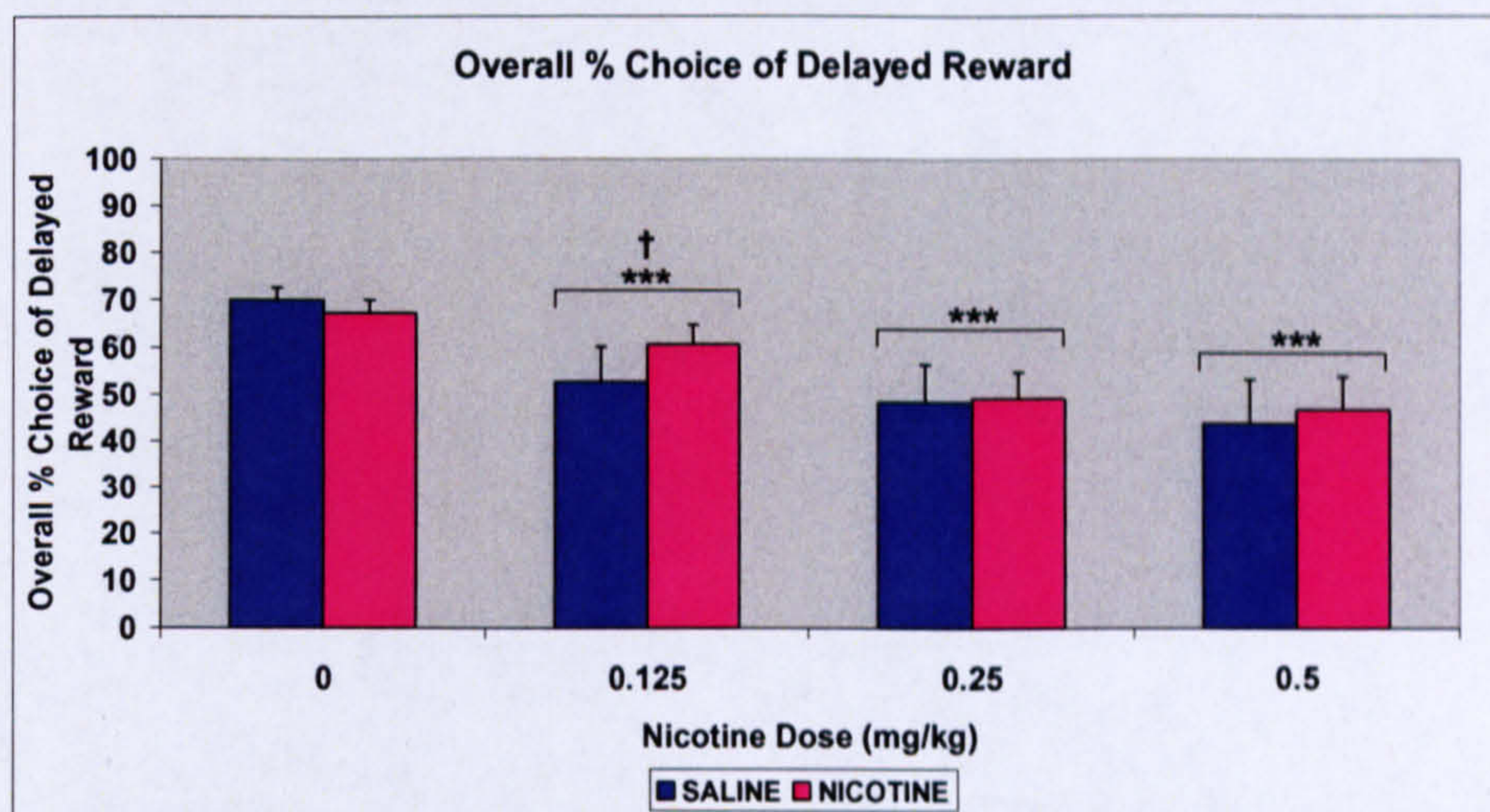


Fig. 8.7: The effects of acute nicotine on overall percentage choice of delayed reward. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5mg/kg nicotine dose.

Analysis of choice by delay once again demonstrated a highly significant reduction of choice of delayed reward ($F(2.078, 45.726) = 20.949, p < 0.001$). Furthermore demonstration of delay dependency was shown, supported by a highly significant main effect of delay ($F(2.206, 48.539) = 154.241, p < 0.001$). Choice of delayed reward decreased significantly with increasing delays, with choice of the delayed reward across all delay conditions differing significantly from choice at other delay trials (all $p < 0.001$). A significant treatment dose x delay interaction was also found, suggesting that the effects of acute nicotine were delay dependent ($F(5.955, 131.016) = 3.171, p = 0.006$). Further analysis of the main effects of treatment at each delay demonstrated, with the exception of 0 seconds, that nicotine promoted choice of the immediate reward (all $F(3,66) \geq 5.228, p \leq 0.002$) (see Fig. 8.8). Post hoc comparisons revealed that the lowest 0.125mg/kg dose, decreased choice of delayed reward during 20 and 40 second

delay trials in comparison to choice during control treatment (all $p < 0.01$). Following 0.25mg/kg, choice of delayed reward was decreased at 10, 20 and 40 second delays in comparison to control. At the highest, 0.5mg/kg dose choice was decreased across the greatest number of delay conditions, reducing significantly choice of delayed reward at 10, 20, 40 and 60 seconds in comparison to saline (all $p < 0.05$) (see Fig. 8.8).

Individual analysis of the main effects of delay across individual nicotine doses revealed that choice was delay sensitive following all treatments (all $F \geq 34.477$, $df \geq 2.490$, 54.785 , $p \leq 0.001$). The delay dependency became greater with increasing doses of nicotine (see Fig. 8.9). Post hoc analysis revealed choice of delayed reward was significantly greater at 10, 20 40 and 60 seconds delays in comparison to the 0 delay condition all doses tested (all $p < 0.01$).

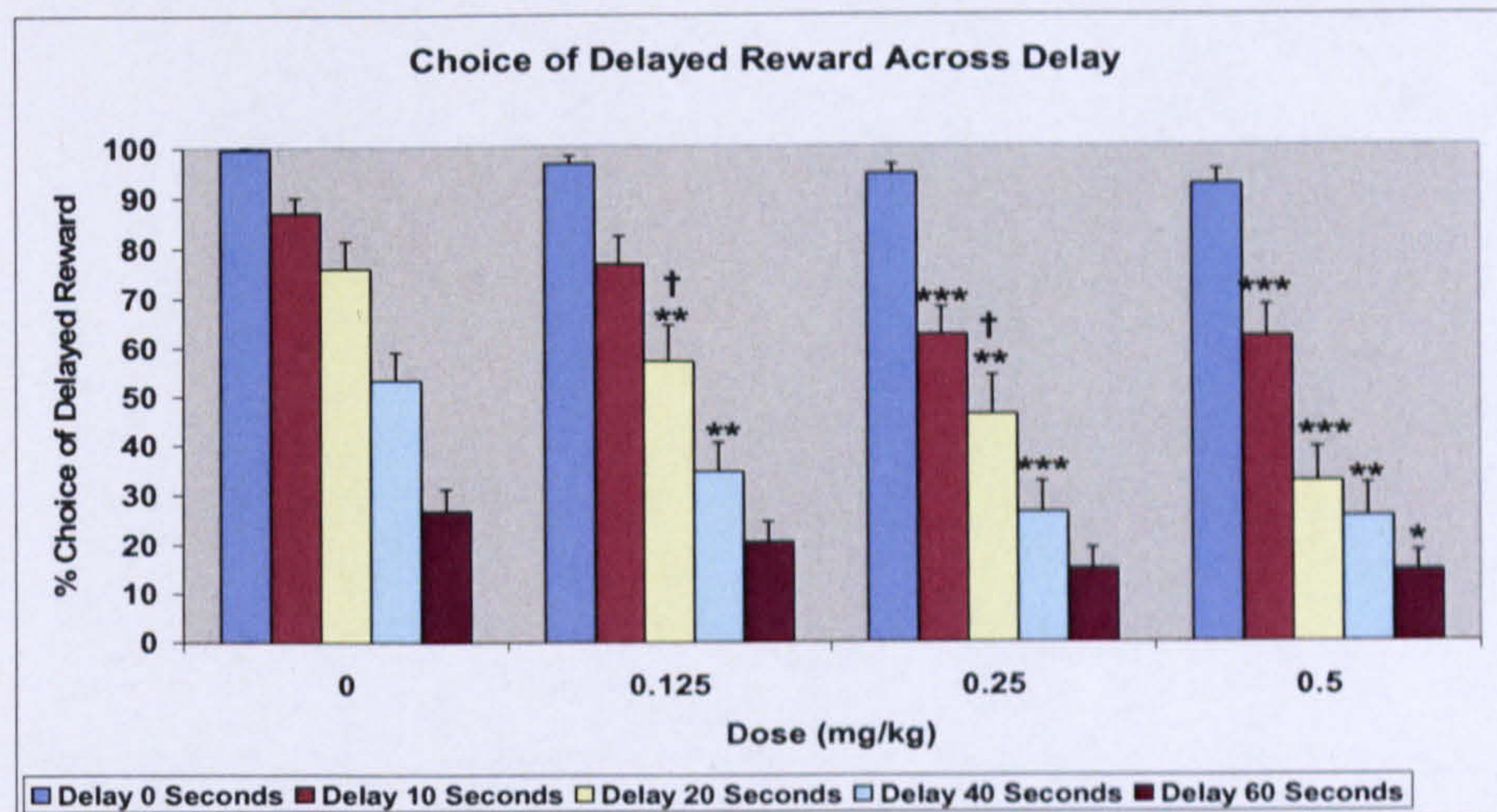


Fig. 8.8: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on percentage choice of delayed reward across each delay condition. Each bar represents the mean percentage \pm SE. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5mg/kg nicotine dose.

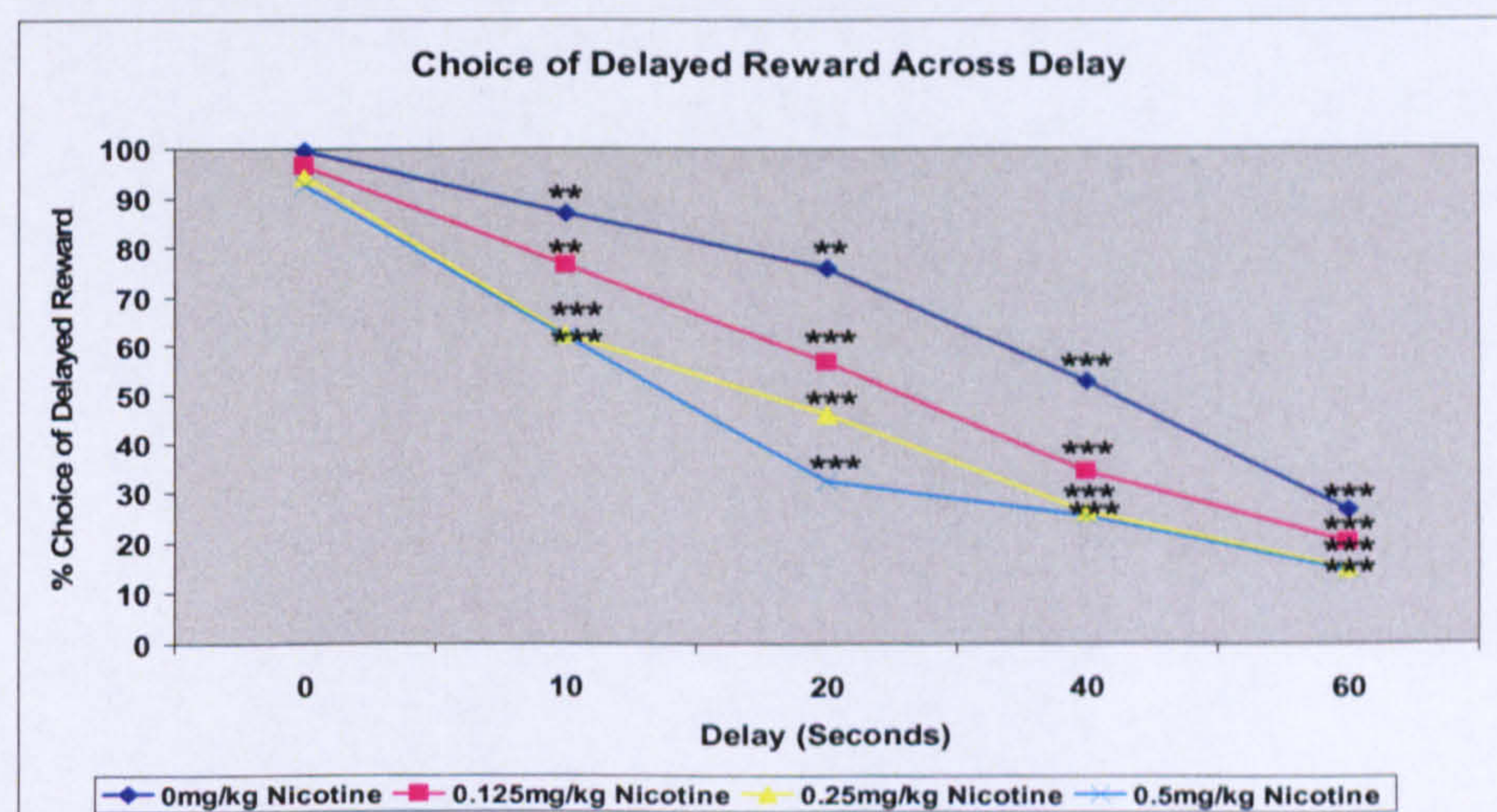


Fig. 8.9: Simple effects analysis of treatment*delay interaction: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

Fig. 8.10(a-d) compare the choice of delayed reward across delays of treatment groups following each dose tested. Almost identical delay dependent choice behaviour was shown following saline treatment, 0.25mg/kg and 0.5mg/kg nicotine doses. Once again, following the 0.125mg/kg dose, evidence of a greater decrease in choice of delayed reward across 10, 20, 40 and 60 second delays was displayed by nicotine naïve animals. Statistically however, no significant differences were demonstrated between treatment groups in response to nicotine. Analysis revealed no significant main effect of group ($F(1,22) = 0.182$, N.S.), group x dose, group x delay or group x dose x delay interaction ($F(2.206, 48.539) = 0.151$, N.S.; $F(2.078, 45.726) = 1.245$, N.S.; $F(5.955, 131.016) = 0.736$, N.S., respectively).

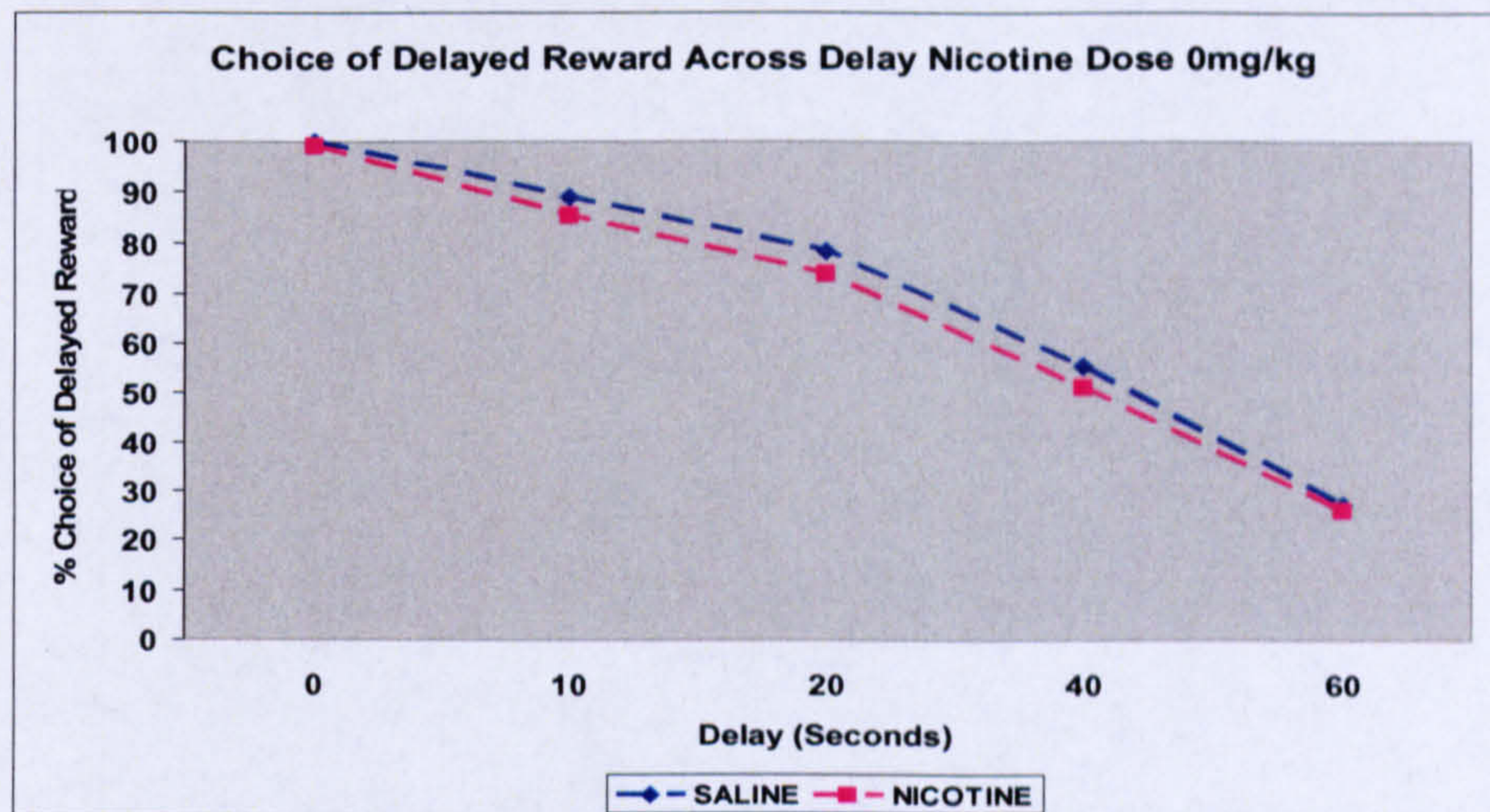


Fig. 8.10(a)

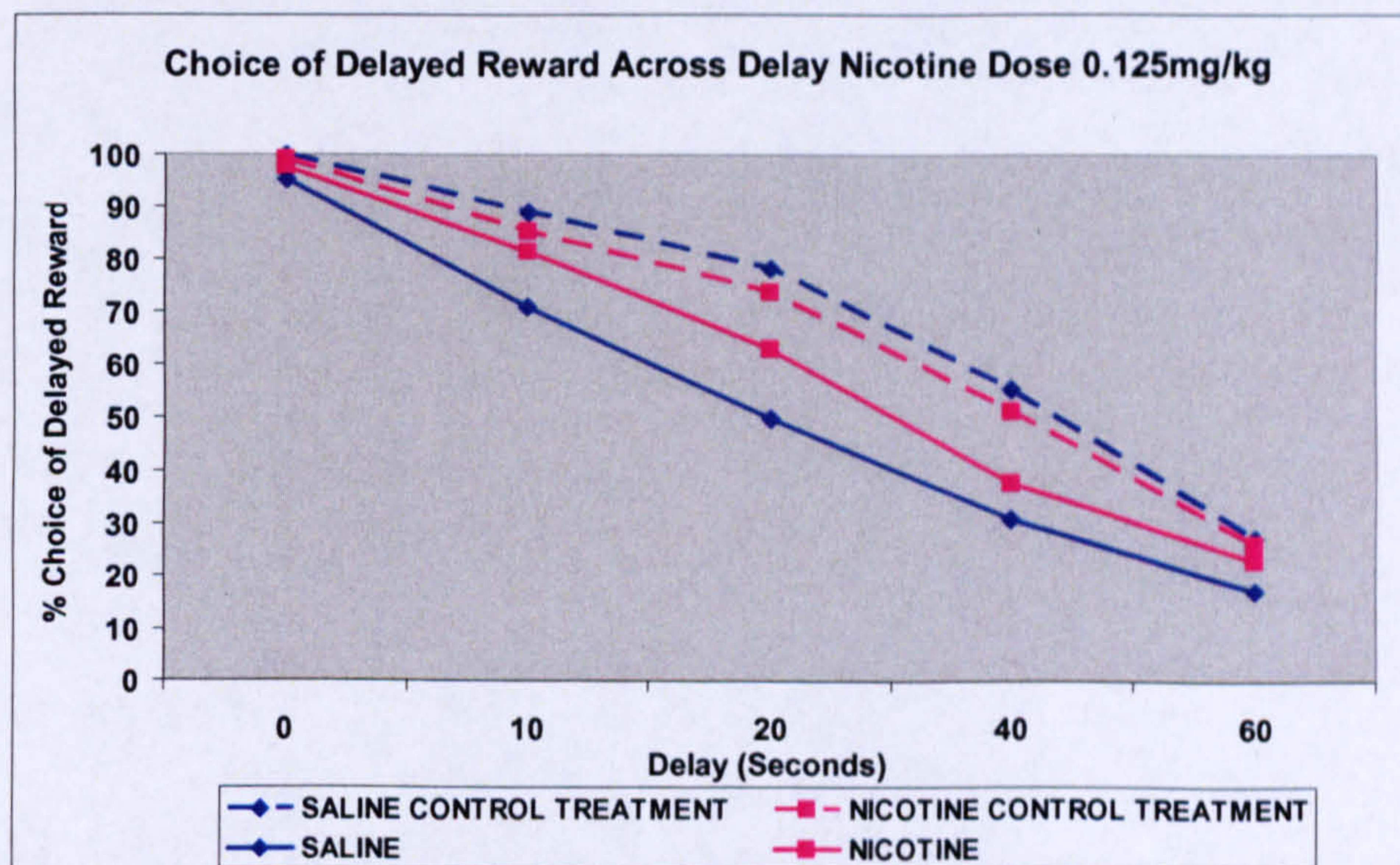


Fig. 8.10(b)

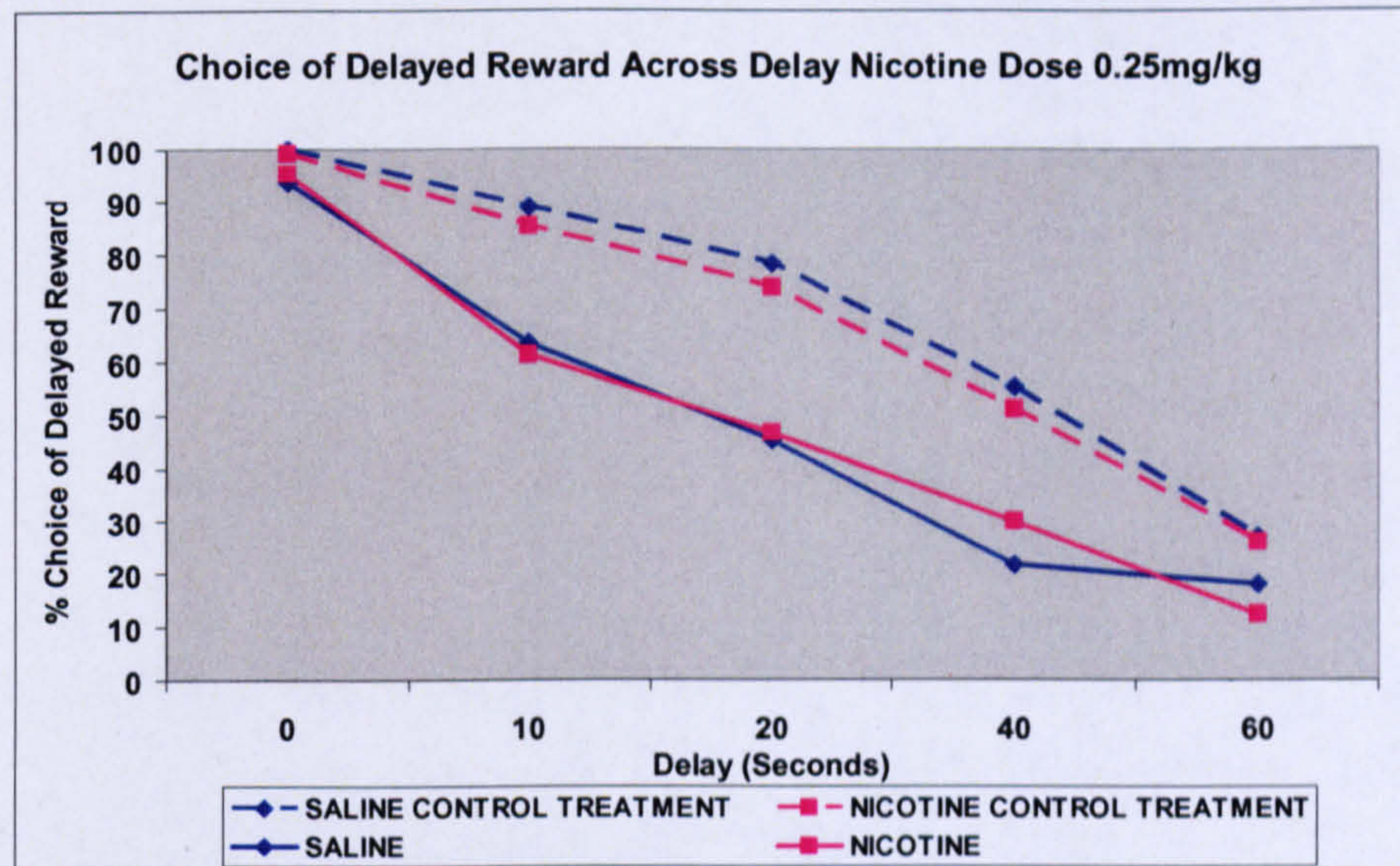


Fig. 8.10(c)

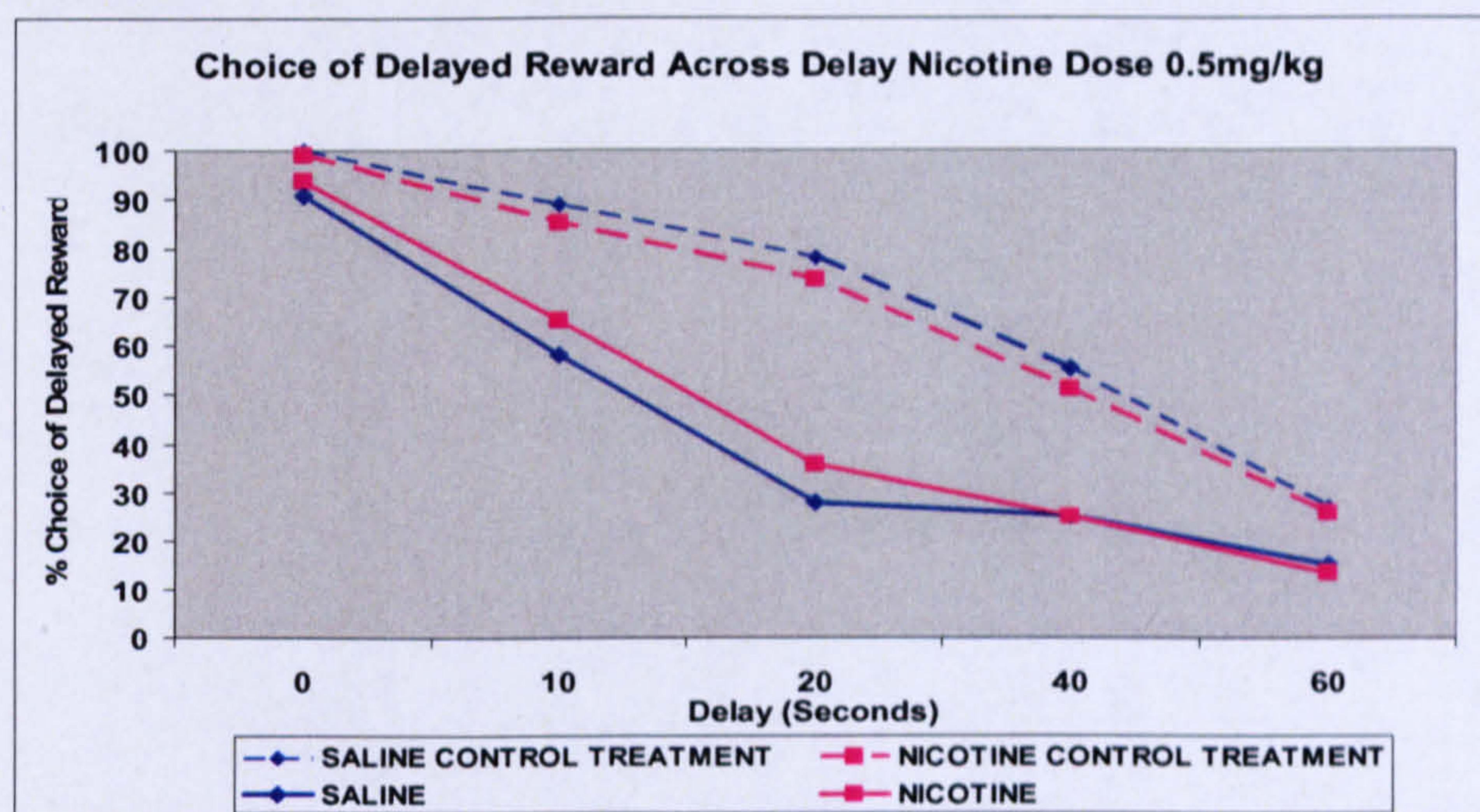


Fig. 8.10(d)

Fig. 8.10(a-d): Choice Behaviour: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

8.3.2.3. Speed of Responding

Table 8.7 summaries speed of responding measures in the task following the administration of acute nicotine challenges. Nicotine treatment did not significantly affect the latency to initiate trials or to choose between the delayed and immediate reward, with an absence of significant main effect of treatment shown across parameters (all $F(3,66) \leq 2.030$, N.S). Furthermore, speed of responding on these measures were comparable across treatment groups with no significant group main effects (all $F(1,22) \leq 0.352$, N.S) or group x treatment dose interactions displayed (all $F(3,66) \leq 2.415$, N.S).

Nicotine differentially affected the latency to collect reward dependent upon the reward choice made. No significant effects treatment were shown on latency to collect reward following an immediate reward choice ($F(2.008, 44.176) = 1.378$, N.S.). In contrast, acute nicotine decreased

the latency to collect the reward following a delayed reward choice in a dose dependent manner ($F(3,66) = 0.444$, $p = 0.020$). Post hoc analysis however failed to demonstrate significant differences between treatment conditions. Comparison of treatment groups once again demonstrated a lack difference in response to nicotine on latency to collect reward, with no significant between group effects (all $F(1,22) \leq 0.560$, N.S) or group x dose interactions shown on both immediate and delayed reward magazine latency ($F(2,008, 44.1760 = 0.576$, N.S; $F(3,66) = 0.444$, N.S., respectively).

Table 8.7: The effect of acute nicotine on speed of responding in the delayed reinforcement task.

Behavioural Measure		Nicotine Dose (mg/kg)			
		Saline (control)	0.125	0.25	0.5
Initiation Latency	N	1.04 ± 0.07	0.96 ± 0.06	0.97 ± 0.10	0.91 ± 0.07
	S	0.92 ± 0.09	0.87 ± 0.05	0.87 ± 0.06	1.01 ± 0.09
Immediate response latency	N	0.66 ± 0.04	0.77 ± 0.05	0.74 ± 0.04	0.77 ± 0.04
	S	0.72 ± 0.04	0.70 ± 0.04	0.72 ± 0.05	0.73 ± 0.05
Delayed response latency	N	0.88 ± 0.11	0.87 ± 0.08	0.85 ± 0.06	0.84 ± 0.06
	S	0.97 ± 0.11	0.85 ± 0.06	0.83 ± 0.06	0.90 ± 0.09
Immediate magazine latency	N	0.20 ± 0.03	0.21 ± 0.02	0.23 ± 0.03	0.22 ± 0.03
	S	0.23 ± 0.03	0.24 ± 0.03	0.22 ± 0.02	0.27 ± 0.03
Delayed magazine latency	N	1.55 ± 0.47	0.92 ± 0.19	0.89 ± 0.20	0.78 ± 0.12
	S	1.60 ± 0.41	1.30 ± 0.31	1.08 ± 0.22	1.04 ± 0.29

Table 8.7: Each value represents the mean latency (seconds) ± SEM.

8.3.2.4. High vs. Low “Trait” Impulsivity

In order to assess differences in responses to acute nicotine challenges based upon baseline levels of “trait” impulsive choice, independent examination of high and low impulsive animals on choice behaviour was in addition conducted.

8.3.2.4.1. Choice Behaviour

Acute administration of nicotine in low impulsive animals reduced the overall percentage choice of the delayed reward ($F(3,39) = 16.906$, $p < 0.001$) (Fig.8.11(a)). The significant increase in sensitivity to delay was observed across all doses tested in comparison to saline control treatment (all $p < 0.01$). Previous nicotine exposure in low impulsive animals had no effect on response to acute nicotine with a lack of both significant between treatment group and group x test day interactions shown ($F(1,13) = 0.006$, N.S.; $F(3,39) = 0.625$, N.S., respectively). Analysis of choice by delay revealed once again a significant main effect of treatment dose ($F(3, 39) = 16.969$, $p < 0.001$). Choice behaviour was furthermore delay dependent ($F(4,52) = 77.336$, $p < 0.001$), with selection of the larger delayed reward decreasing significantly with increasing delay (all $p < 0.01$).

The ability of acute nicotine to decrease choice of the larger delayed reward was furthermore dependent on delay, as indicated by the significant dose x delay interaction ($F(12, 156) = 2.747$, $p = 0.002$). Further analysis of treatment at each delay condition revealed that alteration in choice was not made following treatment during the 0 second delay condition ($F(3,39) = 1.906$, N.S.). Nicotine increased the choice of the immediate smaller reward during the 10, 20, 40 and 60 delay conditions (all $F(3,39) \geq 5.757$, $p \leq 0.002$) (see Fig. 8.12(a)). In comparison to saline treatment, the lowest 0.125mg/kg dose decreased choice of the delayed reward during the 40 second delay condition only ($p < 0.05$). Following 0.25mg/kg nicotine treatment preference for the delayed reward was significantly reduced at both 10 and 40 second delays (all $p < 0.05$). The most profound decrease in choice of delayed reward was observed following the highest 0.5mg/kg dose, where a decreased in choice in comparison to control was indicated during 10, 20, 40 and 60 second delay conditions (all $p < 0.01$).

Analysis of the main effect of delay at each dose further supported the dose dependent increase in impulsive choice. As illustrated in Fig. 8.13(a) under the influence of each treatment dose a main effect of delay was displayed (all $F(4,52) \geq 23.444$, $p \leq 0.001$). Delay dependency became greater with increasing dose of nicotine. Following the administration of saline and the lowest 0.125mg/kg dose, choice of delayed reward only decreased significantly in comparison to 0 delay condition during 40 and 60 second delay trials (all $p < 0.000$). In contrast, at the higher 0.25 and 0.5mg/kg doses, nicotine choice ratios became significantly reduced across all delays

relative to the 0 delay condition (all $p < 0.05$).

Once again, analysis of choice by delay revealed that previous chronic nicotine exposure had no significant effect on the response to acute nicotine challenges in low impulsive animals. Choice behaviour across delays was almost identical in both groups following nicotine treatment (see Fig. 8.14(a-d)). Analysis indicated no significant main effect of group, group \times delay, group \times dose or group \times dose \times delay interactions ($F(1,13) = 0.008$, N.S.; $F(4,52) = 0.152$, N.S., $F(3,38) = 0.808$, N.S.; $F(12,156) = 0.621$, N.S., respectively).

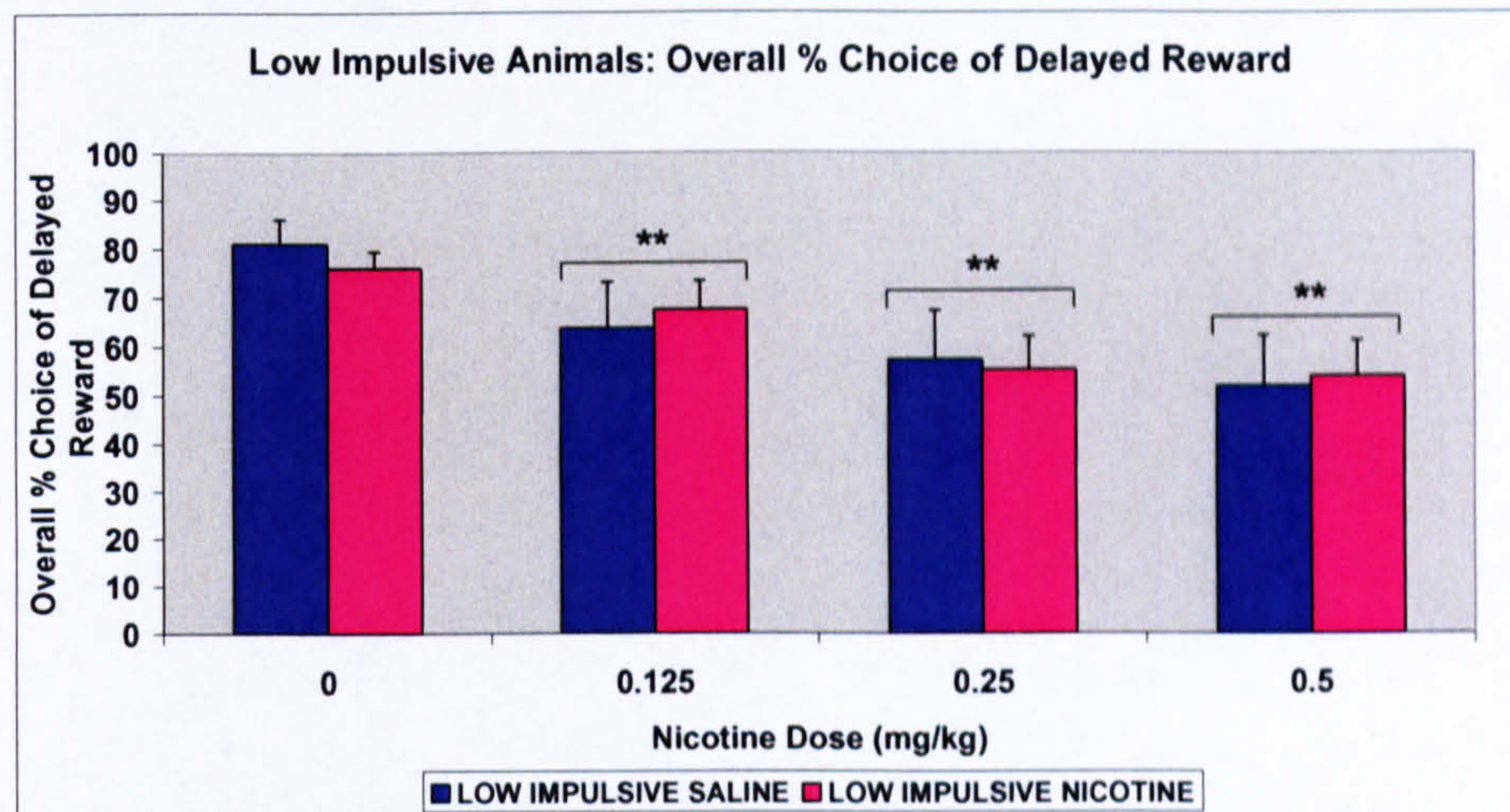


Fig. 8.11(a)

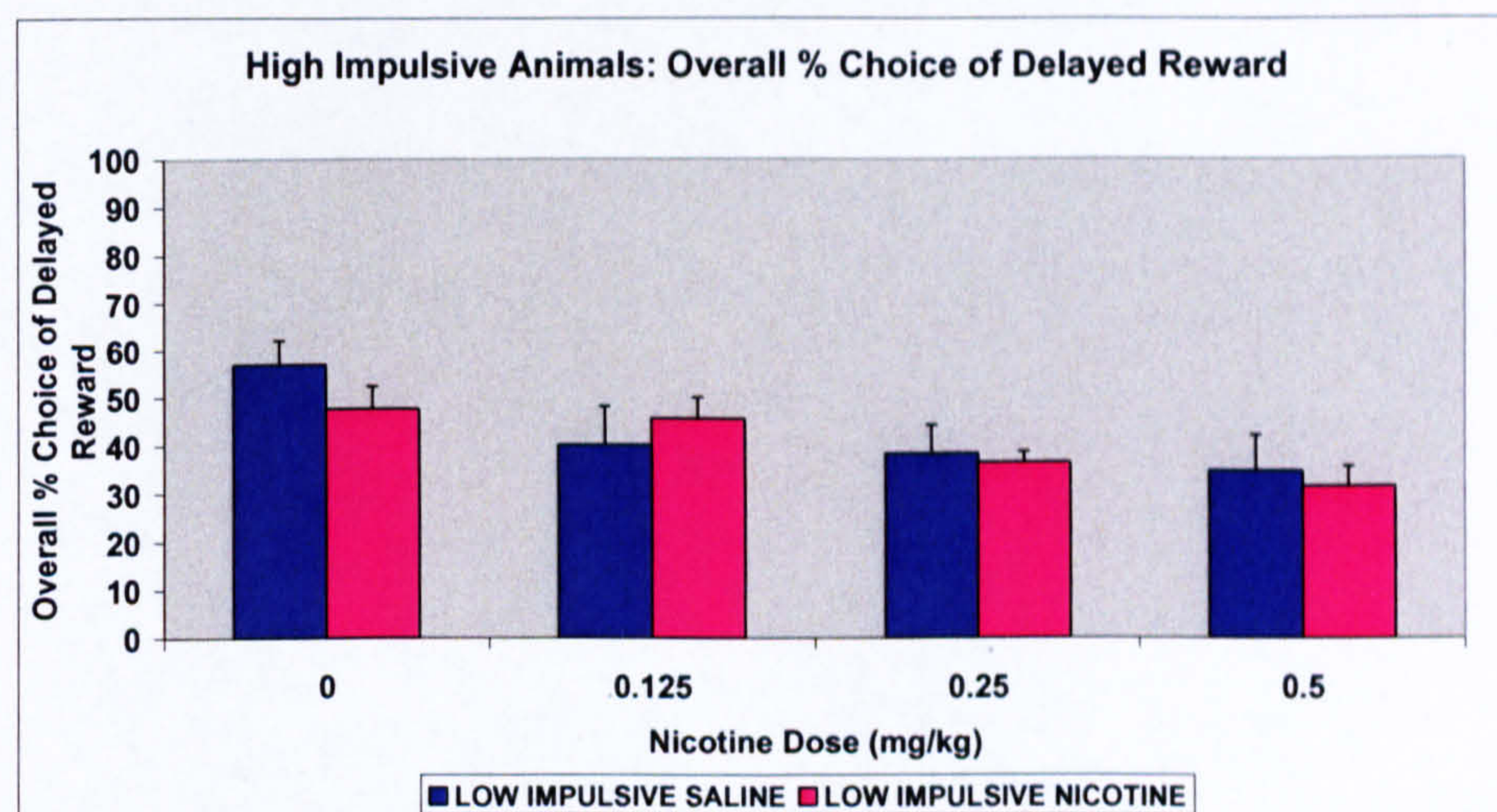


Fig. 8.11(b)

Fig. 8.11 (a-b): The effects of acute nicotine on overall percentage choice of delayed reward in low (a) and high impulsive (b) animals. Each bar represents the mean percentage \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5mg/kg nicotine dose.

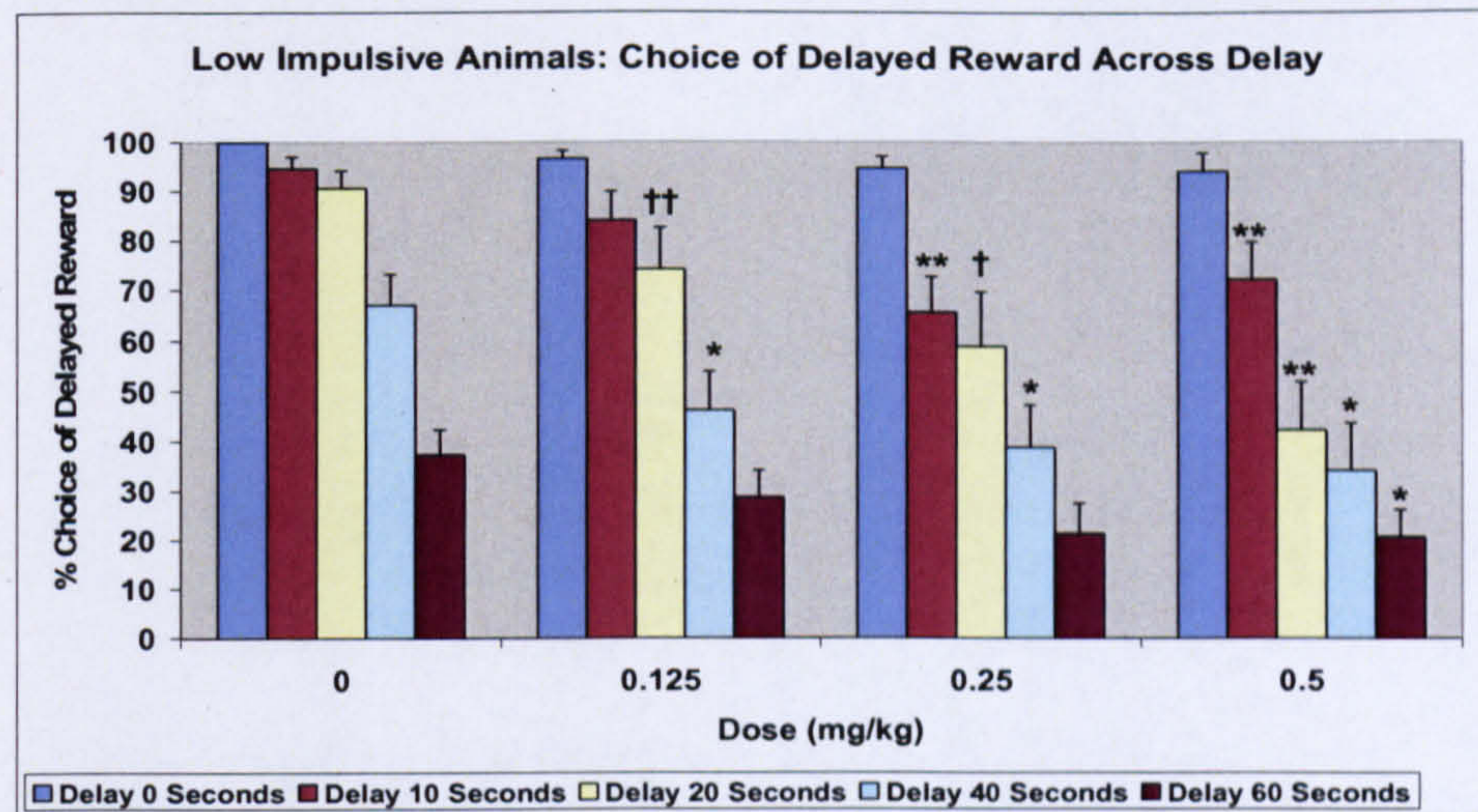


Fig. 8.12(a)

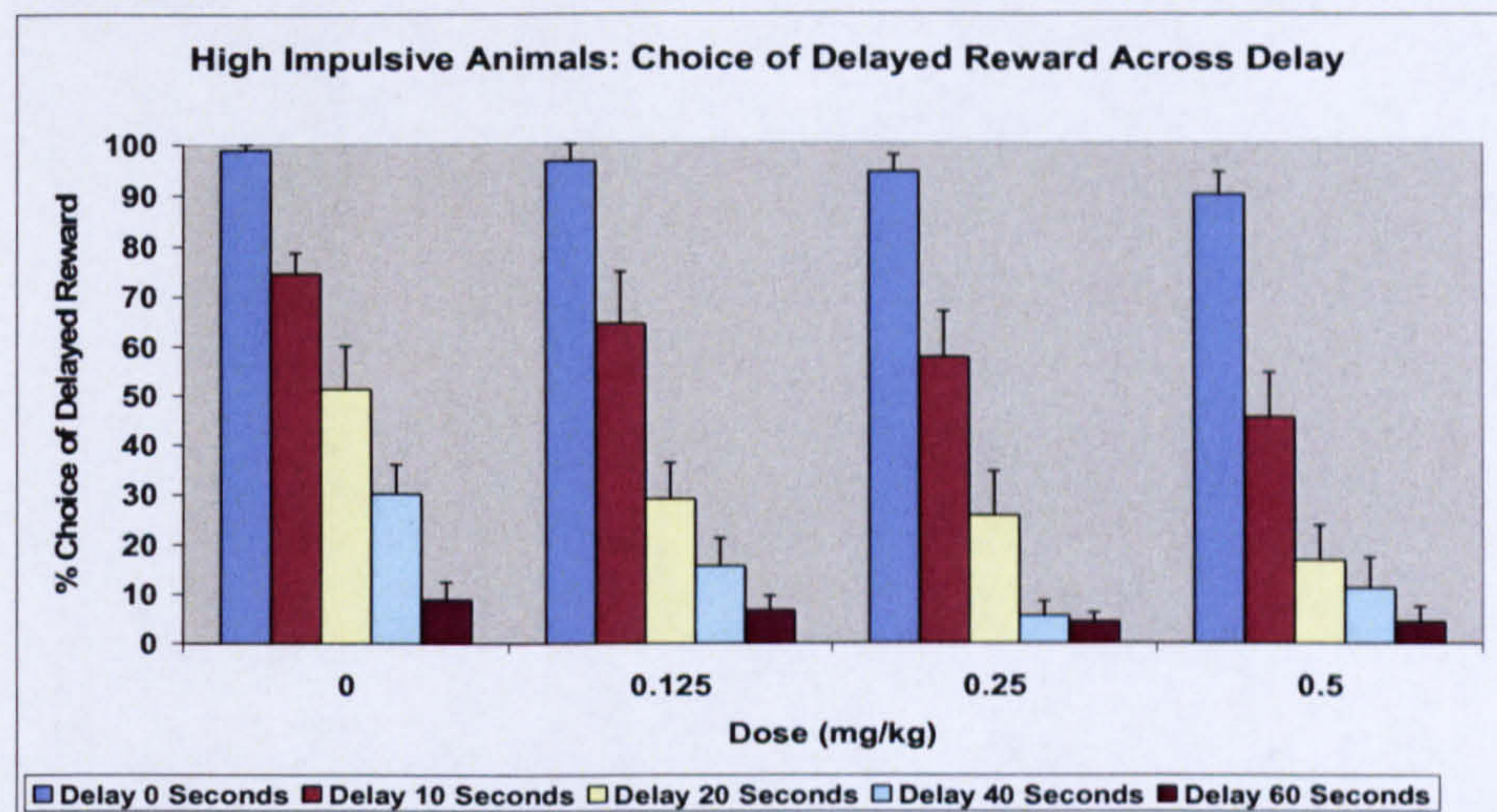


Fig. 8.12(b)

Fig. 8.12(a-b): Treatment*delay interaction: The effects of acute nicotine on percentage choice of delayed reward across each delay condition in low (b) and high (b) impulsive animals. Each bar represents the mean percentage \pm SE. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to saline control. †, $p < 0.05$, ††, $p < 0.01$, †††, $p < 0.001$ (Bonferroni comparison) as compared to highest 0.5mg/kg nicotine dose.

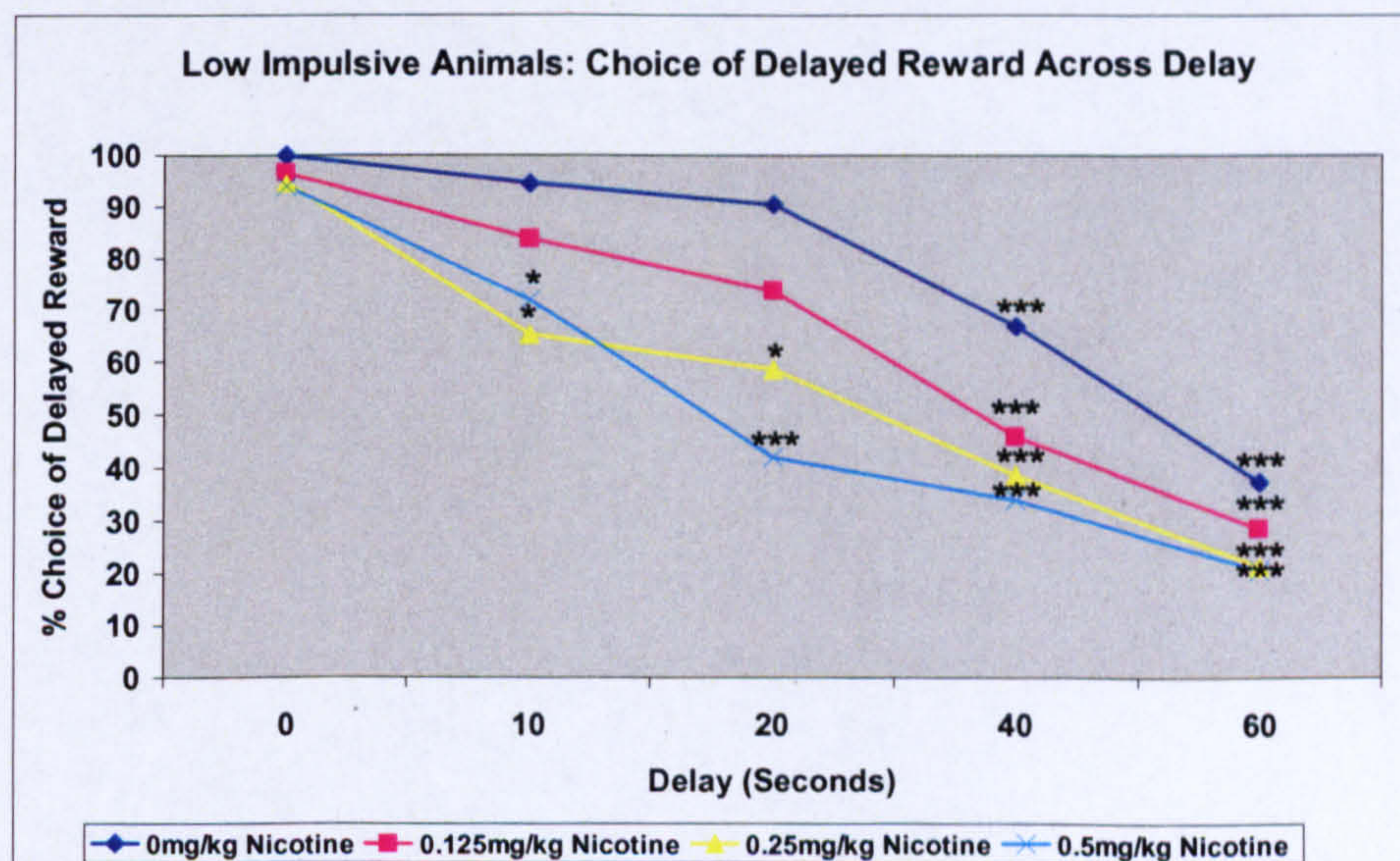


Fig. 8.13(a)

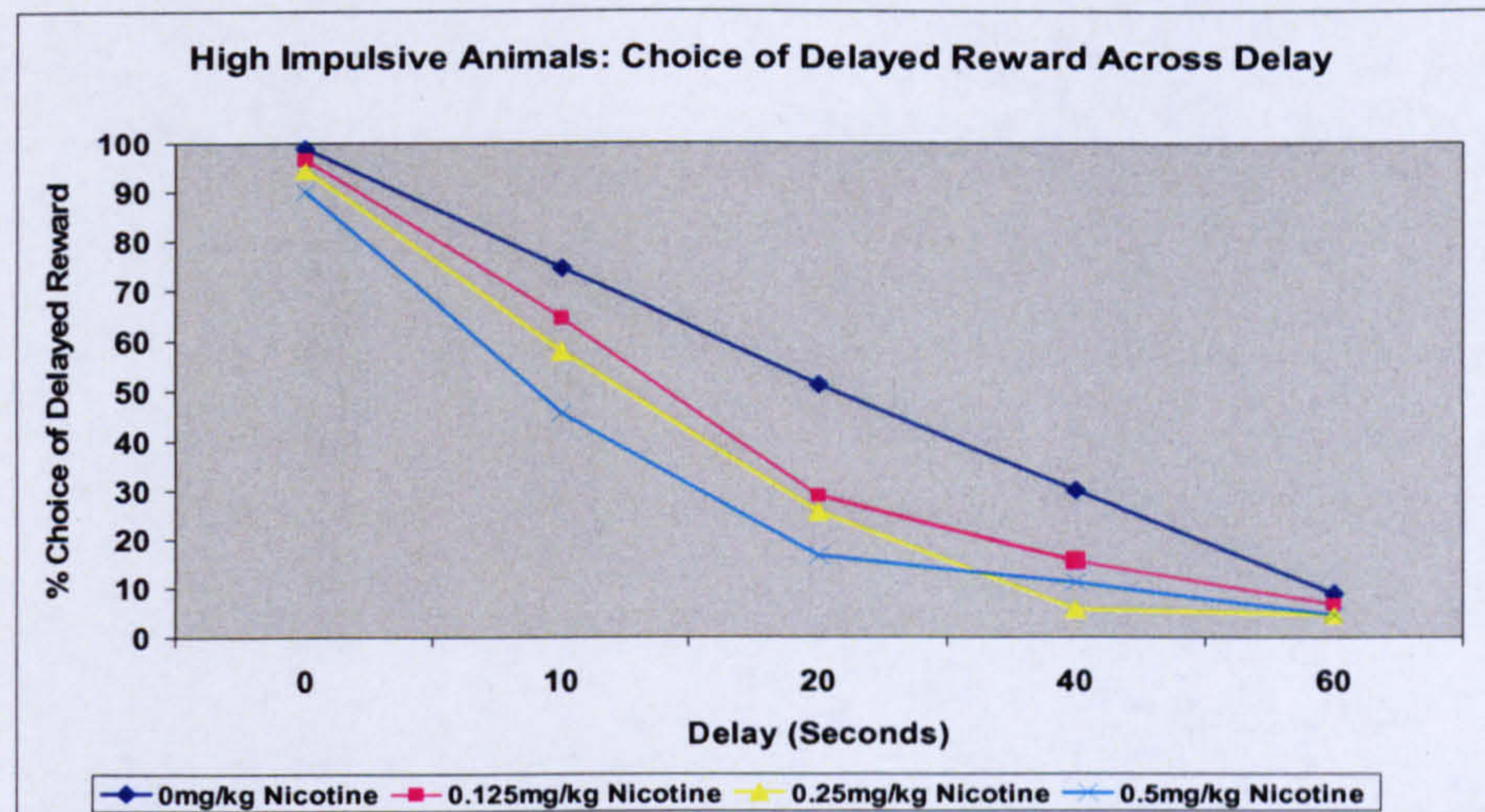


Fig. 8.13(b)

Fig. 8.13 (a-b): Treatment*delay interaction: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose. Each point represents the mean. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ (Bonferroni comparison) as compared to choice of delayed reward at 0 seconds delay condition.

Exploration of the effects of acute nicotine in high impulsive animals demonstrated a decreased choice of delayed reward relative to saline treatment (see Fig. 8.11(b)). Irrespective of treatment group, the effect of acute nicotine on overall choice behaviour was however considerably less dramatic than that observed in the low “trait” impulsive animals. Due to the low sample size of highly impulsive animals, statistical analysis could not be conducted on the data. Comparison of treatment groups revealed that animals with previous nicotine exposure demonstrated a lesser reduction in overall choice of delayed reward following acute nicotine. This differentiation in response is clearly shown at the lowest 0.125mg/kg dose (see Fig. 8.11(b)).

Fig. 8.12(b) examines choice behaviour across delay in high impulsive animals irrespective of treatment group. Clearly evident is the decrease in choice of delayed reward with increasing doses of nicotine across the 10, 20, 40 and 60 seconds delay conditions in comparison to choice behaviour during saline drug treatment. Alteration in choice behaviour was importantly not shown during the 0 seconds delay, where choice of the larger reward was at its greatest. Further evidence of the delay dependent reduction of the delayed larger reward is demonstrated in Fig. 8.13(b). Illustrated is a clear shift leftwards of the delay discounting function, with increasing doses of nicotine. Comparison of the delay dependent choice behaviour of treatment groups is illustrated in Fig. 8.14 (a-d). Mean data once again suggests evidence of a decreased sensitivity to acute nicotine’s ability to decrease choice of delayed reward in animals previously exposed to nicotine. With exception of the 0 delay condition, across treatment doses nicotine naïve animals displayed a greater reduction in choice of the delayed reward across delays following nicotine. The variation across treatment groups in response to acute nicotine is most evident following the treatment of 0.125mg/kg treatment dose.

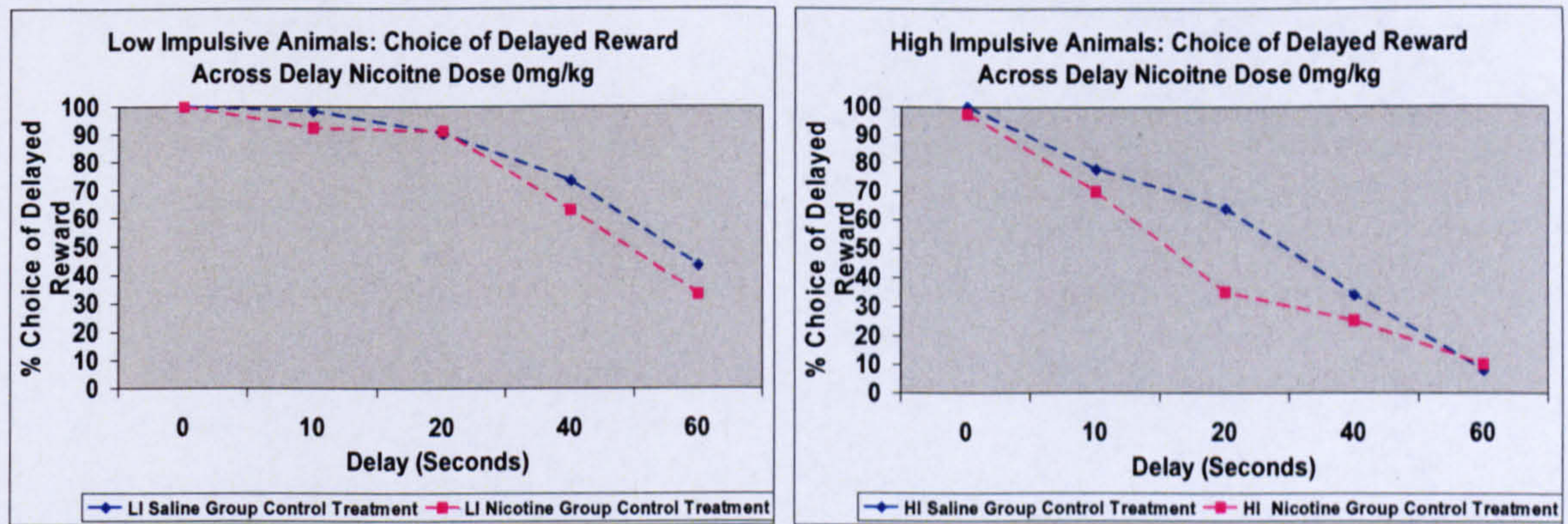


Fig. 8.14(a)

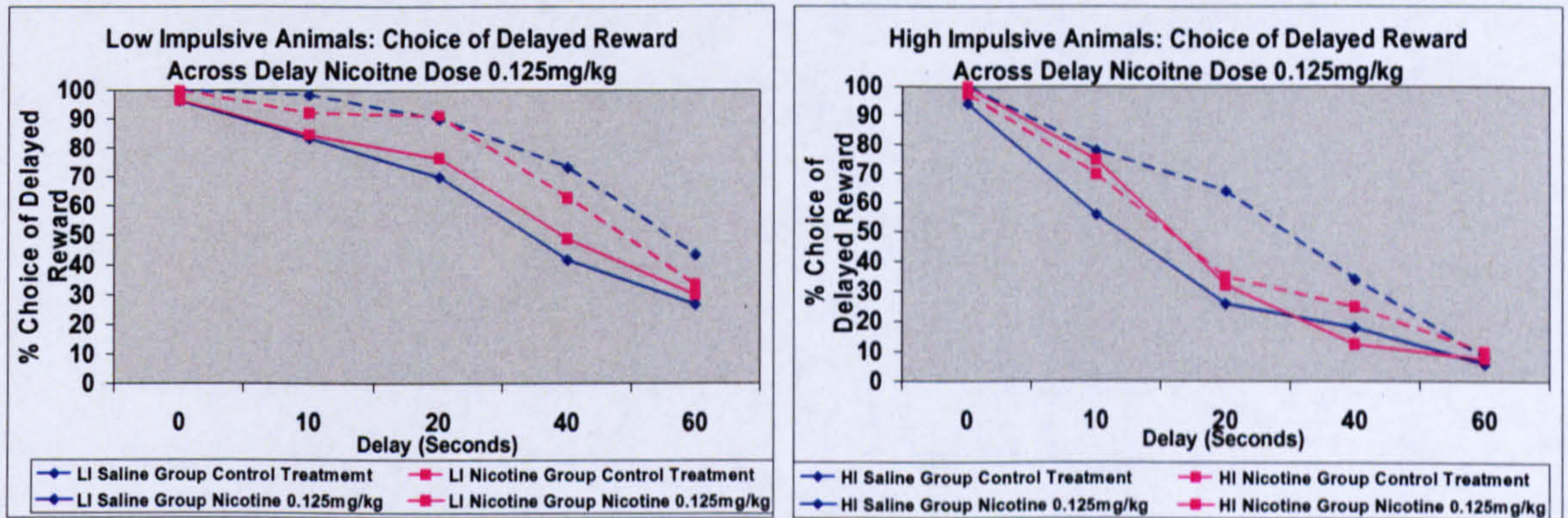


Fig. 8.14(b)

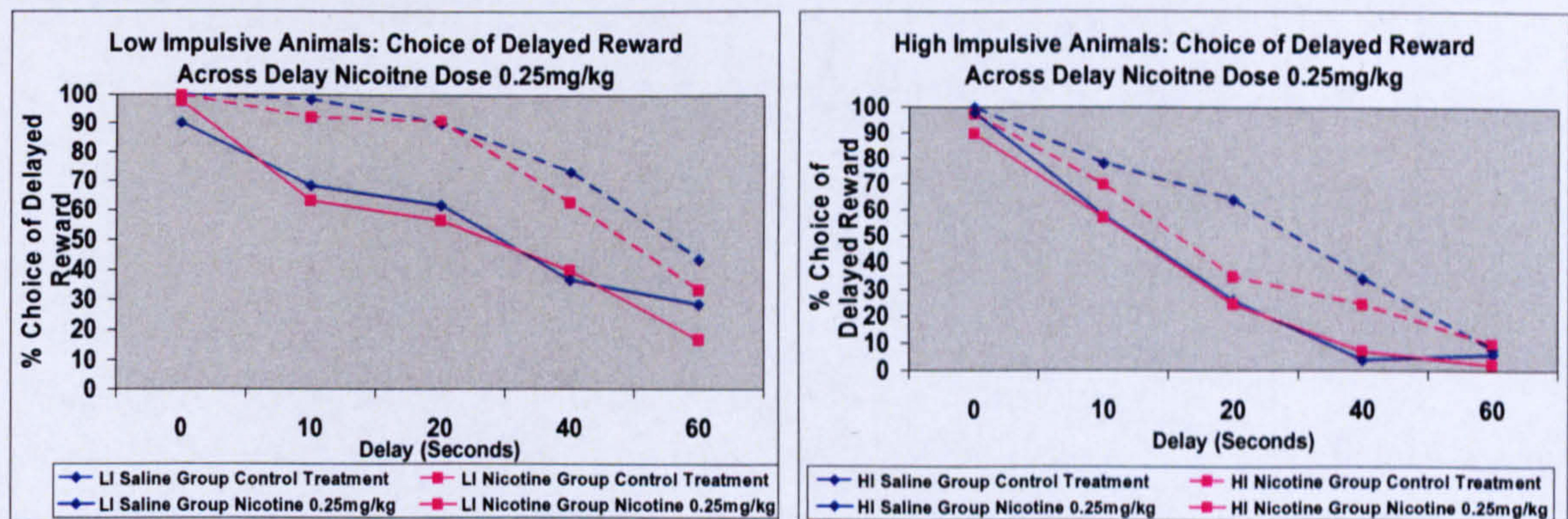


Fig. 8.14(c)

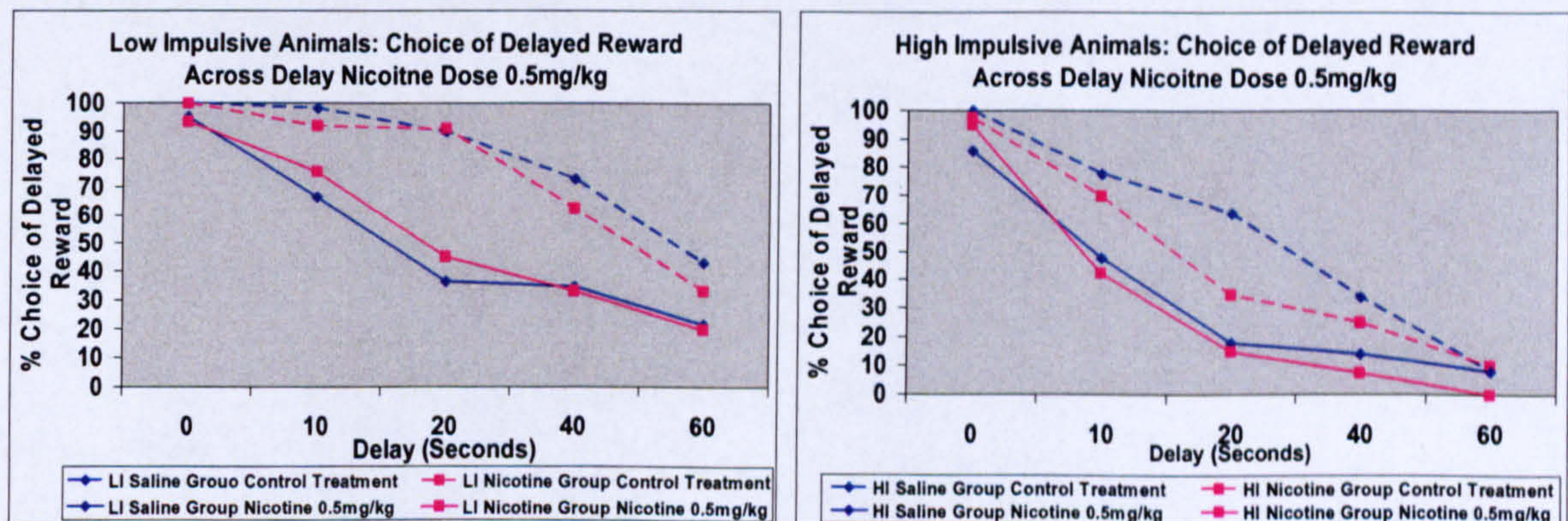


Fig. 8.14(d)

Fig. 8.14 (a-d): Choice Behaviour: The effects of acute nicotine on rate of discounting of delayed reward under each treatment dose in high and low impulsive animals. Each point represents the mean.

8.4. DISCUSSION

Despite there being no doubt of the association between heavy smokers and heightened impulsive choice, an understanding of the complex nature of this relationship remains to be fully determined (Bickel et al., 1999; Mitchell, 1999; Reynolds et al., 2004; Reynolds, 2006a; 2006b; Johnson et al., 2007). The experiments of the present chapter were therefore designed to begin to elucidate more precisely the association between impulsive choice and nicotine dependence. Through use of a longitudinal design, the effects of chronic nicotine, nicotine withdrawal and the residual sensitivity to nicotine following a sustained period of abstinence were assessed on levels of impulsive choice. The data suggested six general conclusions: (1) chronic nicotine exposure is associated with increased impulsive choice, (2) initial nicotine deprivation was without effect on impulsive choice, (3) longer term withdrawal was associated with a substantial increase of impulsive choice, (4) chronic nicotine induced effects on impulsive choice are temporarily transient, (5) previous nicotine exposure appears to have a minimal effect on subsequent response to acute nicotine following a period of sustained abstinence, and (6) high and low “trait” impulsive animals appear to respond differentially to the effects of chronic nicotine and nicotine withdrawal. Each of these key findings will be discussed in detail in the following sections.

8.4.1. Chronic Effects of Nicotine on Impulsive Choice

The chronic administration of nicotine led to a significant reduction in overall choice of delayed reward in comparison to saline treated animals, an effect that was at its greatest during the early stages of drug treatment. Although the effect on choice behaviour was not statistically delay dependent, a clear trend for a decrease in choice of delayed reward was observed across the higher 40 and 60 second delay conditions whilst choice at the 0 second delay condition remained unchanged (see Fig. 8.3(a-d)). Chronic nicotine treatment displayed no other significant effects on performance measures in the task.

These findings suggest that nicotine increased levels of impulsive choice during chronic exposure. The data appear to be consistent with previous research examining the chronic effects of not only nicotine, but also amphetamine and cocaine on levels of impulsive choice in animal models of delayed reward (Richards et al., 1999; Paine et al., 2003; Dalley and Locey, 2005). The present results provide further evidence that the dysfunctional decision making in heavy smokers arises as consequence of nicotine exposure (Bickel et al., 1999; Mitchell, 1999; Reynolds et al., 2004; Reynolds, 2006a; 2006b; Johnson et al., 2007). There are, however, alternative interpretations of these results. The decrease in choice of delayed reward could have been mediated instead by changes in perceived magnitude of reward, or a response bias on the lever delivering the immediate reward (Ho et al., 1999). However, these interpretations of the present effects of nicotine are unlikely for reasons previously detailed in Chapter 7 (section 7.4).

These include the lack sensitivity of animal's choice behaviour to changes in magnitude of reward and more crucially the continued choice of the larger reward during the zero delay condition on almost 100% of trials (Richards et al., 1997; Grace et al., 1999; Farrar et al., 2003; Green et al., 2004). The latter of these reasons support not only animals continued ability to effectively discriminate between different magnitudes of reward, but also provides evidence of a lack of response bias on the immediate lever during chronic treatment.

Alternatively, it could be that the increased choice of immediate reward could have instead been governed by nicotine induced changes in appetite (McNair and Bryson, 1983; Grunberg et al., 1986; Levin et al., 1987; Blaha et al., 1998; Miyata et al., 1999; Zhang et al., 2001). However, this explanation cannot easily account for the present data. Consistent with previous reports, the findings of Chapter 6 indicated that decreasing motivation for food reward over a seven day period did not affect choice in the delayed reward task (see also Cardinal et al., 2000). Furthermore, latency to collect reward, which can arguably be taken as an index of motivation, remained unchanged during chronic drug treatment.

As discussed in the previous chapter two processes that may influence choice behaviour in the delayed reward task are working memory and time perception (Hinson et al., 2003; Mitchell, 2004). Due to the reported positive effects of nicotine on working memory it is unlikely that the increase in preference for the immediate reward was mediated by drug induced impairments in this cognitive process (Rusted and Trawley, 2006; Spinelli et al., 2006). However, as with the acute effects of nicotine on impulsive choice, the ability to overestimate the passage of time is likely to have played a role in the observed alterations in choice behaviour (Pradhan and Dutta, 1970; Bizot, 1997; Carrasco et al., 1998). Nicotine may have led to the delay to the delivery of the larger reward being perceived as longer than the saline treatment group, causing the delayed reward to be discounted to a greater degree. This interpretation does not suggest that the observed alterations in choice behaviour are not indicative of an increase in sensitivity to delayed gratification, but rather that a mechanism by which this may be increased is via a disruption in timing ability.

From reviewing the available evidence, it appears most likely, that chronic nicotine led to an increased sensitivity to delayed reward, rendering animals more impulsive in their decision making. Although nicotine treated animals' choice of delayed reward was consistently lower than that of the control group during treatment, it is important to highlight that the greatest deficit was indicated during the first test session. Following this time point, a gradual increase in self control was observed during the following days of drug treatment (see Fig. 8.1). Although this was not supported statistically by a significant day x group interaction, the observation that nicotine's initial effects were not sustained across the testing period suggest that tolerance to the

effects of nicotine on impulsive choice may have been shown. These findings are comparable to data reported by Paine et al. (2003) who demonstrated induced tolerance the effects of cocaine on delay gratification during a two week drug regime. As discussed previously in Chapter 5 (section 5.5.1), it is well established that tolerance to nicotine's actions develops on a range of behaviours in both human smokers and animals; an effect argued to be mediated by the deactivation and upregulation of central nicotinic receptors (Collins et al., 1990; Wonnacott, 1990; Stoleran, 1999; DiChiara, 2000; Littleton, 2001; Perkins, 2002; Cepeda-Benito et al., 2006; Robinson et al., 2007). As clear demonstration has been made that nicotine mediates its effects on impulsive choice via central nAChRs (see Chapter 7), it is possible that the neural adaptations observed at these receptors could mediate the development of tolerance to nicotine's initial deficit on self control in the delayed reward task.

The neurobiological systems downstream from the nAChRs, that may have been involved in the nicotine induced changes in choice behaviour, have been touched upon in the previous chapter. It is likely that the decrease in choice of the delayed reward is attributable to the enhancement of DA and 5-HT in regions of the NAc and OPFC, as both these neurochemical systems and anatomical regions have been implicated in impulsive choice (Oades and Halliday, 1987; Di Chiara and Imperato, 1988; Benwell and Balfour, 1992; Ribeiro et al., 1993; Nisell et al., 1996; Cardinal et al., 2001; Kheramin et al., 2002; 2004 Mobini et al., 2002; Winstanely et al., 2004; Pothuizen et al., 2005; van den Bergh et al., 2006). Interestingly, with repeated nicotine exposure, the increase in synaptic DA in regions of the VTA and its associated projections begins to decline (Ramussen and Czachura, 1995; Cardoni and Di Chiara, 2000). This effect, which is believed to be in part mediated by the up-regulation of nAChRs, may have therefore played a role in the observed tolerance to the effect of nicotine on impulsive choice (Watkins et al., 2000a). However, as the present behavioural study did not evaluate the neuronal processes involved in the observed effects, the proposed mechanisms remain highly speculative.

8.4.2. Effects of Initial Nicotine Withdrawal on Impulsive Choice

The termination of nicotine treatment caused a spontaneous nicotine abstinence syndrome. This was supported by the marked elevation of somatic symptomatology, beginning at 6 hours post pump removal and remaining elevated for a time period of 133.5 hours (see Fig.8.7). Consistent with previous research the profile of abstinence signs predominately included, gasps, writes, teeth chattering, chews, shakes, tremors, scratches and foot licks (Malin et al., 1992; Hildebrand et al., 1997; 1999; Epping-Jordan et al., 1998; Watkins et al., 2000a; Harrison et al., 2001; Malin et al., 2006). The most frequently observed sign was that of scratches and foot licks, incorporated here within the miscellaneous category. Despite comparable drug treatment regimes, the abstinence syndrome observed in the present research, once again, appears to be of a longer duration than that reported in previous studies. As discussed previously, the most likely

explanation of the inconsistency is the differences in rat strains utilised across studies (Malin et al., 1992; Epping-Jordan et al., 1998; Hildebrand et al., 1999; 1997).

Initial drug withdrawal was associated with no differences in overall choice of delayed reward in comparison to saline treatment group. Choice of the larger reward across delays was also similar across groups. As shown in Fig. 8.1 however, from 108 hours post cessation of treatment a clear trend of a gradual decrease in percentage choice of delayed reward was observed. During this stage animals experiencing nicotine withdrawal were significantly faster at selecting the delayed reward in comparison to saline treatment group. Caution should however be given when interpreting this effect, due to the coincident slowing of response latency relative to BL displayed in saline treated animals.

In comparison to human research the lack of effect displayed during initial withdrawal is consistent with Mitchell (2004) who reported no effects of 24 hours nicotine deprivation on monetary delayed reward tasks in smokers. Although subjects discounted cigarettes to a greater degree during drug withdrawal, authors concluded that this was attributed to an increase in preference for cigarettes rather than enhanced sensitivity to delay. The current data are, however, in opposition to a more recent study reporting greater levels of discounting of delayed reward in smokers who had abstained for a minimum of 13 hours (Field et al., 2006). The most likely explanation for differences in findings across studies is the use of real versus hypothetical rewards. More comparable to the present research, the subjects in Mitchell (2004) made choice regarding real rewards and experienced the delay to their delivery. In contrast, in the study conducted by Field et al., hypothetical rewards were instead utilised during the task.

If the lack of effect of early nicotine withdrawal observed in both the present research and Mitchell (2004) are valid results, the implications of these findings is that impulsive choice may not be crucially involved in relapse to smoking during early abstinence. Instead the aversive symptoms of withdrawal, or intense craving, may be key factors involved in the failure to remain abstinent during this stage (e.g. Koob and LeMoal, 2001; Shiffman et al., 2003; Bagot, Heishman and Moolchan, 2007; Hughes 2007b).

It is important to consider when interpreting these findings that withdrawal from chronic nicotine has been associated with an increase in appetite and weight gain (Grunberg et al., 1986; 1987; Miyata et al., 1999). Consistent with previous research, studies in Chapter 6 demonstrated that increasing motivation for food reward both acutely and for a duration of 7 days, led to a marginal increase in the overall choice of delayed reward in the paradigm (Bradshaw and Szabadi, 1992; Wogar 1992; Ho et al., 1997). It is possible that the changes in the value of the reward may have masked nicotine's effects on increasing impulsivity. Indeed, nicotine

deprivation is associated with deficits in DA functioning in both the mesolimbic system and NAc (Fung et al., 1996; Hildebrand et al., 1998; Watkins et al., 2000; Koob and Le Moal, 2001; Rahman et al., 2004). As inhibiting DA neurotransmission in the NAc and related regions of BLA, has been associated with the heightening of impulsive choice (Cardinal et al., 2001; Winstanley et al., 2004a; Denk et al., 2005), the lack of difference between groups on levels of impulsive choice during nicotine withdrawal appear somewhat inconsistent with neurobiological data. However, the effects of nicotine on motivation for food reward in the task is likely to have been minimal, as no evidence of alterations in latency to collect reward were demonstrated. As will later be discussed, differences in high and low trait impulsivity in response to nicotine withdrawal are likely to have masked the effects of nicotine deprivation on impulsive choice at this stage.

8.4.3. Effects of Long Term Nicotine Withdrawal on Impulsive Choice

The gradual decrease in the overall choice of the delayed reward that began at 108 hours post termination of treatment, continued and reached significance during the second week of nicotine withdrawal. During this stage of abstinence, animals experiencing nicotine withdrawal consistently selected the delayed larger reward to a lesser extent than the saline treated animals. The effect was furthermore delay related, with preference of the delayed reward being significantly lower during the 40 second delay trials. By the third week of nicotine withdrawal the nicotine withdrawal induced impulsivity had recovered, and choice behaviour in chronically nicotine treated animals was indistinguishable from that of the saline group. During withdrawal week two and week three, both treatment groups performed comparably on speed of responding measures in the delayed reward task.

These findings suggest that week two of withdrawal is associated with an increase in levels of impulsive choice. Based on the present findings, it can be predicted that smokers may be more likely select the immediate rewarding effects of nicotine due to the delayed larger rewards associated with continued abstinence being discounted to a high degree during this stage. As discussed previously (see section 5.43.), 75% of smokers relapse within the first two weeks of abstinence (Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995). The present findings suggest that impulsivity may, therefore, be an important aspect mediating the high rates of relapse during “quitting” attempts in smokers. This is further supported by recent findings in human research demonstrating that high levels of discounting of delayed reward significantly predicted relapse in smokers (Dallery and Raiff, 2007; Krishnan-Sarin et al., 2007; Yoon et al., 2007). Taken together, strong evidence has been provided that impulsive choice may be strongly associated with abstinence success. Pharmacological and behavioural treatment interventions that focus upon decreasing levels of impulsive choice during this “critical period” of nicotine withdrawal may therefore prove to be effective future interventions

for smoking cessation.

The neuronal changes that have developed as a consequence of chronic nicotine exposure are likely to have mediated the heightened impulsivity during this stage of abstinence. Although it cannot be determined from the present research what neurobiological processes are involved, results from brain imaging studies have demonstrated evidence of structural deficits in regions implicated in impulsive choice. For example, smokers have demonstrated reduced grey matter volumes and density in frontal regions, including the ACC, and PFC and OPFC (Gallinat et al., 2006). Whilst it cannot be determined conclusively whether these abnormalities preceded drug use, or were instead a consequence of drug exposure, strong evidence for the latter of these theories was provided by the demonstrated inverse correlation between years of abuse and frontal volume. Abnormalities within these regions appear furthermore to persist following cessation of smoking, with abstinent smokers displaying significant hypoactivation in the PFC, in particular the orbitofrontal cortical areas (Neuhaus et al., 2006). Whilst the ACC and PFC appear to play little role in the regulation of impulsive choice (Cardinal et al., 2001), strong evidence has implicated the sub-region of the OPFC in delay discounting processes (Kheramin et al., 2002; 2004; Mobini et al., 2002; Winstanley et al., 2004). It is reasonable to suggest, therefore, that abnormalities within the OPFC may play key role in the mediation of the transient heightened impulsive choice observed in the present research in the absence of nicotine.

The neuronal changes involved in the mediation of heightened impulsivity during withdrawal, appear to however be temporary. By the third week of withdrawal, chronically nicotine treated animals had returned to levels of delay discounting that were similar to that of the saline treatment group. These data support and extend the findings of Dallery and Locey (2005), where following the termination of 65 days of chronic nicotine treatment animals remained more impulsive for approximately four weeks before a return to BL levels was shown. The present data, when considered with the results reported by Dallery and Locey (2005), suggest that with longer duration of nicotine exposure, the time course required for the CNS to mediate the strengthening of self control following termination of treatment will be greater. This research supports the findings of Bickel and colleagues (1999), where smokers displayed considerable greater levels of impulsive choice than both ex-smokers and non-smokers, whom displayed indistinguishable levels of discounting. As pre-baseline levels of impulsivity were not recorded in the study it could not be determined whether low levels of impulsive choice had enabled the ex-smokers to successfully abstain, or conversely whether impulsivity was a reversible effect of nicotine dependence. Based on the present research findings clear support has been provided for the latter of these theories.

8.4.4. Effects of Acute Nicotine Challenges on Impulsive Choice in Animals Previously Exposed to Chronic Nicotine

Three weeks following the cessation of chronic nicotine treatment the effects of a series of acute nicotine challenges was assessed on levels of impulsive choice. Previous nicotine exposure had no significant effect on the response to acute nicotine on choice behaviour in the delayed reward task. Acute nicotine in both the nicotine and saline animal's markedly reduced overall choice of delayed reward in a dose related manner, with all doses differing significantly from saline treatment. The effect moreover was delay dependent, with no significant alterations in choice observed at the 0 second delay trials across all doses tested. Instead, with increasing doses of nicotine a greater reduction in preference for the larger reward was observed across delay conditions, with the highest dose reducing choice significantly at 10, 20, 40 and 60 second delay trials. No significant difference between treatments groups was furthermore observed in response to nicotine on speed of responding measures in the task. Nicotine had no significant effect on latencies to initiate trials, select rewards or collect rewards following an immediate choice. However, acute nicotine treatment decreased latency to collect reward following a delayed reward choice, although post hoc analysis failed to locate a significant difference between doses.

In support of the findings of the previous chapter, these data suggest that acute nicotine can substantially increase levels of impulsive choice. This effect was observed in both drug naïve animals and in animals previously exposed to chronic nicotine. However, no differences in choice behaviour were observed between groups, suggesting that chronic nicotine exposure did not affect subsequent response to nicotine following a three week period of abstinence. Whether alterations in the responses to nicotine on impulsive choice would have been observed during earlier stages of abstinence is unknown. It could be the case that the neuronal changes resulting from chronic exposure that render animals more sensitive to the affects of nicotine on impulsive choice, recover rapidly following cessation of treatment. It is essential that future research examines the affects of nicotine at earlier stages of abstinence to determine whether this is indeed the case.

8.4.5. High Verses Low “Trait” Impulsivity: Differences in Response to Chronic Nicotine, Nicotine Withdrawal and Acute Nicotine Following Abstinence

Consistent with both the findings of Chapter 6 and previous research, extensive BL variability in levels of impulsive choice was indicated across animals (e.g. Cardinal et al., 2000; Winstanley et al., 2003a; Deltu-Hagedorn, 2006). Once again the delay discounting function was well described by the exponential model. Based on estimates of k , a group high ($k: 0.5420 \pm 0.07$) and low impulsive animals ($k: 0.2130 \pm 0.03$) were identified. To determine whether differences in trait impulsivity influenced the response to nicotine on impulsive choice; choice

behaviour of high and low impulsive animals were independently examined.

Assessment of choice behaviour of low impulsive animals revealed that chronic administration of nicotine led to a substantial decrease in overall choice of delayed reward. Once again the greatest reduction was observed during the first test session. This effect was delay dependent, with a decrease in preference for the larger delayed reward in comparison to choice behaviour of saline treated animals at 10, 20 and 60 second delay conditions. Cessation of drug treatment was associated with continued high levels of impulsive choice during withdrawal week one and week two. A decrease in choice of the larger reward was observed at 60 second and 40 and 60 second delay trials, during withdrawal week one and week two respectively. This significant difference between treatment groups during withdrawal was aided by the coincident slight increased choice of delayed reward in saline treated animals in comparison to BL choice. The nicotine induced impulsive choice gradually returned to levels similar to that of the saline treatment group by the 17th day post termination of treatment.

Consistent with previous analysis, there was no significant effect of long term nicotine on the subsequent responsivity to acute nicotine following a three week period of abstinence. Low impulsive saline and nicotine treated animals both displayed a significant reduction in overall choice of delayed reward across all doses tested in comparison to saline control treatment (0.125mg/kg, 0.25mg/kg and 0.5mg/kg). The effect was also delay dependent, with no significant alteration in choice behaviour during the 0 delay trials. With increasing doses of nicotine, a greater reduction in preference for the delayed reward was observed across the 10, 20, 40 and 60 second delay conditions. Once again, the highest nicotine dose reduced choice of the larger reward significantly across all delay trials with the exception of the 0 second delay condition.

In contrast, high impulsive animals displayed no evidence of alterations in choice behaviour during chronic drug treatment. In direct opposition to the low impulsive group, during initial drug withdrawal an increase in choice of delayed reward from 36 to 84 hours of nicotine withdrawal was displayed. This effect was most prominent at the 20, 40 and 60 second delay conditions. The enhanced choice of delayed reward was short lived and by the second week of withdrawal no differences between treatment groups were evident on task performance. Conversely, during the third week of withdrawal evidence of a lower choice of delayed reward in comparison to the control animals was indicated between 17 and 20 days post cessation of treatment. The difference in reward preference across groups was at its greatest at 10 and 20 second delay conditions. However, the difference in choice behaviour treatment groups appears to be due to the substantial increase in preference of the delayed reward displayed by saline treated animals relative to BL performance. Conversely, the choice behaviour expressed by high

impulsive animals in nicotine withdrawal displayed minimal deviation from BL. Caution should therefore be given when interpreting these findings as evidence of nicotine induced changes in impulsive choice. By 21 days post termination of drug treatment, high impulsive saline and nicotine treated animals displayed comparable levels of choice behaviour.

Acute nicotine challenges decreased overall choice of delayed reward in the task, although to a much lesser degree than the low impulsive group. The greatest reduction in preference for the larger reward was indicated at the 20 and 40 second delay trials. In further contrast to the low impulsive group, a greater response to acute nicotine was observed in drug naïve animals in comparison to animals previously exposed to chronic nicotine. The apparent difference in response between treatment groups was most evident at the lowest 0.125mg/kg dose, where the animals chronically treated with nicotine animals displayed minimal alterations in choice behaviour in comparison to the choice behaviour following the control dose (Fig. 8.14(b)). This preliminary finding may suggest that neural adaptations associated with long term nicotine exposure may have rendered high impulsive animals more tolerant to the effects of nicotine on impulsive choice.

Taken together, these findings are the first of preclinical research to suggest that low impulsive animals may be more sensitive to the effects of nicotine induced intolerance to delayed gratification. It is highly unlikely that the high impulsive group were insensitive to the effects of nicotine due to a floor effect. The overall choice of delayed reward of high impulsive animals ranged between 50-60%, therefore enabling a reduction in overall choice behaviour to still be observed. Indeed following acute nicotine administration, choice of the delayed has been shown in some animals to be reduced to below 15%. The variation in response to nicotine is instead most likely due to differences in the underlying neurobiology of high and low impulsive animals. Research for example has demonstrated that more impulsive humans and animals display lower levels of 5-HT functioning (McGuire and Raleigh, 1987; Roy et al., 1988; Westergaard et al., 1999). Furthermore, lower D2 receptor availability has been associated with heightened impulsivity, as measured by levels of inhibitory control (Dalley et al., 2007). The effects of nicotine may therefore differ in high impulsive animals due to the drug acting upon an already dysfunctional neurobiological system. Indirect support for this theory has been provided by a recent study by Oswald et al. (2007). In their study high impulsivity was associated with a blunted DA release in the right VS following the administration of amphetamine. As both DA and regions of the VS have been strongly implicated in the mediation of impulsive choice, the differences in response to chronic nicotine may be the result of the less substantial DA release in this region in high impulsive animals (e.g. Cardinal et al., 2000; Winstanley et al., 2004a; Pothuizen et al., 2005; Helms et al., 2006).

Interestingly, spontaneous nicotine withdrawal resulted in opposite effects on impulsivity in high and low impulsive animals. This most likely accounts for the lack of significant differences between saline and nicotine treated animals when the analysis was conducted regardless of “trait” impulsivity in week one of withdrawal (see section 8.4.2). The heightened impulsivity in low impulsive animals during nicotine deprivation is more consistent with previous preclinical findings which indicated evidence of persistent heightened impulsive choice following the termination of chronic nicotine treatment in rodents (Dallery and Locey, 2005). Furthermore, the response of low impulsive animals is in agreement with the majority of human studies reporting an enhancement of intolerance to delayed reward in drug deprived smokers and opiate addicts (Giordano et al., 2002; Field et al., 2006). Taken together, these findings suggest that during initial nicotine withdrawal, impulsivity may be a key process underlying relapse in individuals who were less impulsive prior to the initiation smoking. The induced heightened intolerance to delay at this stage of abstinence may increase the likelihood of these individuals selecting the more immediate reinforcing effects of nicotine, or relief withdrawal symptoms, over the delayed rewards associated with a drug free lifestyle.

The finding that high impulsive animals display a short lived improvement in self controlled decision making, is once again most likely attributable to differences between high and low impulsive in the underlying neurobiology on which nicotine acts (McGuire and Raleigh, 1987; Roy et al., 1988; Westergaard et al., 1999; Dalley et al., 2007). Interestingly, these findings also compare, to some extent, to that of a recent study demonstrating a decrease in levels of impulsive disinhibition in high impulsive animals following withdrawal from self administered cocaine (Dalley et al., 2007). These findings suggest that initial nicotine deprivation may be associated with greater levels of self control over drug taking behaviour in highly impulsive individuals. It is important to note however, that despite the evidence of an increase in self control at this stage of withdrawal, levels of impulsivity still remained higher than that of low impulsive animals.

The heightened impulsive choice continued in low impulsive subjects until the 17th day post termination of treatment. These neural adaptations associated with chronic nicotine exposure however did not affect the response to acute nicotine in low impulsive animals following the return to BL performance. Conversely, in high impulsive animals neuronal changes associated with chronic nicotine exposure appeared to have a minimal effect on levels impulsivity during longer term withdrawal. However, evidence of a developed tolerance to the subsequent acute administration of nicotine was however displayed. These findings provide further support that drugs of abuse, such as nicotine, may be affecting the neurobiology of high and low impulsive individuals differentially. The tolerance to nicotine in this group is most likely a reflection of the up regulation of nAChRs which can persist for up to a month following cessation of treatment

(Trauth et al., 1999). The up-regulation in nAChRs is believed to lead to a decline in nicotine induced DA release. (Ramussen and Czachura, 1995; Cardoni and Di Chiara, 2000). If high “trait” impulsivity is indeed associated with a blunted DA response to drugs of abuse, than tolerance to nicotine may have further exacerbated this abnormality (Oswald et al., 2007). Consequently this could have led to the high impulsive animals to more likely reflect the nicotine induced tolerance to the effects of nicotine at this stage of abstinence.

From preliminary explorations conducted, strong evidence has been presented that high and low trait impulsive animals differentially respond to the effects of chronic nicotine and nicotine withdrawal. In general, low impulsive animals appear more sensitive to nicotine induced sensitivity to delayed reward. These findings suggest that the heightened impulsivity in smokers may only be a consequence of chronic nicotine exposure in individuals who exhibited low “trait” impulsiveness prior to the initiation of smoking. Once individuals have begun smoking, the effect of nicotine and nicotine withdrawal on impulsive choice may be a fundamental process in the maintenance and relapse of smoking behaviour in these individuals. As high impulsive rodents appear to display minimal a response to chronic nicotine and nicotine withdrawal, a “trait” preference of immediate over delayed gratification may instead be more crucially a risk factor in the initiation of smoking in high impulsive individuals. In support of this theory high impulsive animals have been found to self administration greater levels of cocaine (Perry et al., 2005) and consume larger amounts of alcohol (Poulos et al., 1995). Once smoking has been initiated, heightened trait impulsivity is likely to continue to be a key aspect in the maintenance of drug taking and relapse. Although evidence of a decrease in impulsive choice was observed during early withdrawal, the level of impulsivity still remained substantially greater than that of low impulsive subjects. This suggests that dysfunctional levels of impulsivity remain, and are therefore likely to continue to contribute relapse during early drug deprivation.

8.4.6. Limitations

A potential limitation of Experiment 7 was that assessment of performance in the delayed reward task only began on the second day of chronic nicotine administration. If testing had been initiated earlier, a more substantial effect of nicotine on impulsive behaviour may have been observed. However, based on experimenter observations it was felt that choice behaviour in the task may be sensitive to the surgical implantation of the osmotic pump. Therefore, the decision to allow animal's greater time to recover from surgery is likely to have increased the validity of the present findings.

It is furthermore important to stress that the observed differences between high and low impulsive animals are based only on preliminary explorations, in particular in relation to choice

behaviour of high impulsive subjects. Due to the low number of identified high impulsive animals this did not permit the statistical analysis of choice data. Therefore conclusions are based solely on trends in the current data and should be approached with caution. The replication of present research with greater subject numbers would address this issue.

8.4.7. Conclusions

In conclusion, the present results are consistent with theory that heightened impulsivity in smokers is in part a consequence of nicotine induced effects of impulsive choice. When chronically administered, nicotine leads to a significant increase in impulsive choice in rodents. With continued drug exposure evidence of a developed tolerance to nicotine's effects was however shown. Cessation of nicotine treatment gave rise to a spontaneous nicotine abstinence syndrome during which a limited effect of drug deprivation was observed on impulsive choice. However, with continued abstinence in week two of withdrawal an increase in impulsive choice was observed. By the third week of withdrawal the nicotine induced effects on impulsivity had recovered, with a return of choice behaviour to the levels comparable to the control group. Finally, chronic nicotine treatment appeared to have a minimal impact on subsequent response to the drug following a sustained period of abstinence. Preliminary exploration of the differences in response to nicotine between high and low impulsive animals tentatively suggests that low impulsive animals may be more sensitive to the effects of chronic nicotine on impulsive choice than high impulsive animals. Furthermore, these animals displayed a persistent intolerance delayed reward following the termination of treatment that continued for a two week period. In contrast, chronic nicotine had a minimal effect on impulsive choice in high impulsive animals and evidence of a slight decrease in impulsive choice was displayed during initial drug withdrawal. This may be related to a blunted DA response that has been reported in high impulsive individuals in response to drugs of abuse (Oswald, 2007). This is further supported by the evident tolerance to acute nicotine in high impulsive animals following a sustained period of abstinence.

Taken together, these findings suggest that nicotine induced impulsive choice may be important in the maintenance of and relapse to smoking, in particular in low "trait" impulsive individuals. Exposure to nicotine during the early stages of addiction will render individuals more sensitive to delay reward, leading to the increased preference of the more immediate reinforcing effects of nicotine over the longer term health benefits associated with a drug free life style. Consequently, the impairment in self control will lead to the continuation of drug taking and an increased likelihood of the transition to and maintenance of nicotine dependence. The neural adaptations that then develop as a consequence of chronic nicotine exposure may render individuals vulnerable to relapse due to the rewards associated with continued abstinence being discounted to a high degree during abstinence. Future research is necessary that investigates the

neurobiological substrates mediating the association between impulsivity and nicotine dependence at these stages of addiction. Such research will facilitate the progression of the development of potential therapeutic agents that may focus on increasing levels of self control in the hope of aiding smoking cessation.

CHAPTER 9

General Discussion

The principal aim of this thesis was to further our understanding of the relationship between impulsivity and addiction. Despite the accumulating evidence of heightened impulsivity across drug abusers, the cross sectional design of the majority of these studies does not permit inferences to be made regarding whether impulsivity is a cause or consequence of drug abuse (Vuchinich and Simpson, 1998; Bickel et al., 1999; Kirby and Petry, 2004; Dom et al., 2006). The experiments of this thesis were primarily concerned with exploring the latter of these theories, in the hope of determining the role of drug induced impulsivity in the establishment, maintenance and relapse of drug dependence.

More specifically, Chapter 3 and Chapter 6 of this thesis involved the validation of two operant models, each measuring a distinct component of impulsivity. The symmetrically reinforced go/no-go conditional visual discrimination task assessed behavioural disinhibition (Harrison et al., 1999), whilst the delayed reward task measured levels of impulsive choice (Evenden and Ryan, 1996; Cardinal et al., 2000). In Chapter 4 and Chapter 7 the effects of acute nicotine on disinhibition and impulsive choice were examined. Furthermore, the role played by the central nicotinic receptors in the mediation of these effects was determined. Experiments reported in Chapter 5 and Chapter 8 examined longitudinally the effects chronic nicotine, nicotine withdrawal, and the residual sensitivity to nicotine following a sustained period of abstinence, on disinhibition and impulsive choice respectively. Finally, Chapter 8 involved the preliminary exploration of the differences in response to chronic nicotine administration, nicotine withdrawal, and subsequent nicotine challenges based upon basal "trait" levels of impulsive choice.

As these experimental chapters included a detailed discussion of the results, this chapter will instead focus upon summarising and more importantly comparing the overall main findings for each aspect of impulsivity. Exploration will then be made of the broader implications of these findings and future directions.

9.1. VALIDATION OF THE SYMMETRICALLY REINFORCED GO/NOGO TASK AND DELAYED REWARD PARADIGM

The control studies of Chapter 3 and Chapter 6 demonstrated the utility of both the

symmetrically reinforced go/no-go task and the delayed reward task as effective models to explore the association between nicotine dependence and impulsivity. Extending previous findings, both paradigms demonstrated long term stability of levels of inhibitory control and impulsive choice (Harrison et al., 1999; Winstanley et al., 2003a; Ohmura et al., 2005; Dalley et al., 2007). Evidence of individual differences in levels of inhibitory control and intolerance to delayed reward was furthermore indicated, although to a much greater extent in the model of delayed reward. The emergence of differences across animals, despite the receipt of identical training, provides strong evidence that the variability in performance at baseline may reflect differences in “trait” level of impulsiveness in these paradigms (Poulos et al., 1995; Perry et al., 2005; Dellu-Hagedorn, 2006; Dalley et al., 2007).

Consistent with previous research, strong evidence was displayed that choice behaviour in the delayed reward task was contingent upon the delay to delivery of reward (Mazur, 1987; Evenden and Ryan, 1996; Cardinal et al., 2000; Isles et al., 2003; Green et al., 2004). Rodents displayed a systematic shift in response from a choice of the larger delayed reward at short delays, to the choice of the smaller immediate reward when the delay to the delivery of the larger reward was increased. The pattern in choice behaviour was highly sensitive to changes in delay, with a reduction in delays leading to an increased preference of the larger reward.

Crucially, the experiments of Chapter 3 furthermore provided evidence that the accuracy of responding in the go/no-go task was not heavily dependent on timing mechanisms. This was supported by the insensitivity of accuracy of responding during both Go and No-go trials to alterations in the duration of the stimulus presented during No-go trials. Animals clearly utilised the exteroceptive cues in the task to inform them when to respond and when not to respond, minimising the need for timing behaviour (Harrison et al., 1999). As nicotine is known to distort the perception of time (e.g. Carrasco et al., 1998), this finding importantly provided an empirical basis for interpreting the effects of nicotine on inhibitory control in later studies.

As it is well established that nicotine has profound effects on appetite, a further objective of these chapters was to provide evidence that the observed alterations in behaviour in future studies were unlikely to be governed by drug induced changes in primary motivation (Grunberg 1982; Grunberg, 1986; Grunberg, Bowen and Winders, 1986; Klesges et al., 1989; Miyata et al., 1999; Pomerleau et al., 2000; Zhang et al., 2001). Both models were shown to be insensitive to a decrease in primary motivation for food reward, achieved through prefeeding prior to the test session. Conversely, increasing motivation for food reward differentially affected the two sub components of impulsivity. Decreasing food intake the day prior to testing led to no alterations in levels of inhibitory control in the go/no-go task. In contrast, consistent with previous research, a decrease in levels of impulsive choice was observed following both acute and long term increases in primary motivation (Bradshaw and Szabadi, 1992; Wogar 1992; Ho et al.,

1997). The contrasting effects of increased deprivation, provide further support for the theory that impulsive choice and inhibitory control are dissociable components of impulsivity (Evenden, 1999). As nicotine withdrawal has been associated with an increase in appetite and weight gain (Grunberg et al, 1986; Miyata et al., 1999), the observed decrease in impulsivity in the delayed reward task highlighted the need for caution when interpreting the effects of nicotine deprivation on task performance in this paradigm.

9.2. ACUTE EFFECTS OF NICOTINE ON IMPULSIVITY AND THE MEDIATING ROLE OF THE CENTRAL NICOTINIC RECEPTORS

If the differences in levels of impulsivity between drug users and non-drug users arise as a consequence of drug exposure, this could be due to the acute pharmacological effects of the drug. As such, it was important to assess the acute effects of nicotine on impulsivity. Acute administration of nicotine led to significant increases in levels of both disinhibition and impulsive choice in rodents. This effect was more pronounced when measures of impulsive choice were considered. Here, a dose related reduction in choice of overall delayed reward was displayed (0.125mg/kg, 0.25mg/kg, and 0.5mg/kg). Conversely, the induced disinhibition in the go/no-go task was only displayed at the 0.5mg/kg dose tested. These findings suggest that when nicotine is administered acutely, different subcomponents of impulsivity may vary in their sensitivity to the drug, with inhibitory control perhaps decreasing only under specific conditions. Indeed, previous research has suggested that nicotine only induces disinhibited responding in the 5CSRTT, at low doses (0.03-0.3mg/kg) and under task conditions of high attentional demand (e.g. Mirza and Stoleran, 1998; Blondel et al., 2000; Stoleran et al., 2000; Mirza and Bright, 2001; Hahn, et al., 2002; Hahn et al., 2003; Bizarro, et al., 2004; Bruin et al., 2006; Day et al., 2007). Conversely, consistent with the present research findings, the single study that has assessed the effects of acute nicotine on sensitivity to delayed reward reported a substantial increase in impulsive choice across a range of doses tested (0.1mg/kg - 1.0mg/kg). Behavioural inactivity on choice behaviour was only displayed at the 0.03mg/kg dose (Dallery and Locey, 2005).

These findings indicate that two distinct and unrelated subcomponents of impulsivity may be increased by nicotine (Ho et al., 1999; Reynolds et al., 2006a; Winstanley et al., 2006; Dom et al., 2007), supporting the theory that the poor inhibitory control and greater preference for immediate over delayed gratification exhibited by smokers may be, in part, a consequence of nicotine exposure (Bickel et al., 1999; Mitchell, 1999; Spinella 2002; Reynolds et al., 2004; Reynolds, 2006a; Johnson et al., 2007; Yakir et al., 2007). As the findings were demonstrated in drug naïve animals, the nicotine induced effects on impulsivity may therefore be a fundamental factor underlying the early stages of addiction. Initial smoking may lead to a loss of control over future drug seeking and taking behaviour and furthermore increase the likelihood of individuals

selecting the more immediate reinforcing effects of nicotine over the larger, delayed health benefits associated with a drug free lifestyle. As a consequence drug taking is likely to continue, thus increasing the likelihood of the transition to dependence.

These findings may also extend to the relationship between impulsivity and dependence in abusers of other addictive substances. Consistent with the present findings, accumulating evidence indicates that drugs of abuse including cocaine, amphetamine, alcohol, THC and MDMA can increase impulsivity in comparable paradigms in both humans and animals (e.g. Cardinal et al., 2000; Fillmore et al., 2002; Isles et al., 2003; Kieres et al., 2004; Mc Donald et al., 2003; Paine and Olmstead, 2004; Hellmans et al., 2005; Cheng et al., 2006; Reynolds et al., 2006b; van Gaalen et al., 2006). Overall findings have not always been consistent, with reports of both a lack of and even a *decrease* in impulsivity found following drug treatment, with factors including dose level, BL impulsivity, the presence of a reward predicting cue and the variation in paradigms used likely to explain the discrepancies across previous research (e.g. Richards et al., 1997; Cardinal et al., 2000; Wade et al., 2000; de Wit et al., 2002; Mc Donald et al., 2003; Fillmore et al., 2005; 2006a). Taken together, the evidence suggests that drug induced impulsivity may be a key aspect mediating the continuation of drug taking behaviour across drug addictive disorders.

The experiments of Chapter 3 and Chapter 6 additionally assessed the role of the central nicotinic receptors in the mediation of nicotine's effects on impulsivity. This was achieved through assessment of the effects of combination drug treatments of nicotine and the centrally acting nicotinic antagonist MEC (Varanda et al., 1985; Martin, 1990; 1989; Francis and Papke, 1996). MEC successfully reversed the effects of nicotine on both impulsive choice and disinhibition, which had no effect on either subcomponent of impulsivity when administered alone. These findings suggest that nicotine enhances both impulsive choice and disinhibition via centrally located nAChRs. However, due to the lack of specificity of MEC, conclusions could not be made regarding the specific nicotinic receptor subtypes that may be involved. Although there is potential evidence from previous research to suggest that the $\alpha 4\beta 2$ and $\alpha 7$ may mediate nicotine induced disinhibition (Blondel et al., 2000; Grottick and Higgins, 2000; Keller et al., 2005; Hoyle et al., 2006), the effect of more selective nicotinic antagonists, such as DH β E, have yet to be investigated in a model of delayed reward. At this stage it cannot be assumed that both subcomponents of impulsivity are mediated by comparable nicotinic subtypes, as impulsive choice and inhibitory control have often demonstrated dissociated relationships with addiction at the receptor subtype level (e.g. Wade et al., 2000; Talpos et al., 2006; van Gaalen et al., 2006).

9.3. CHRONIC EFFECTS OF NICOTINE ON DISINHIBITION AND IMPULSIVE CHOICE

A main objective of this thesis was to adopt chronic long term research designs that could afford causal interpretation of the relationship between impulsivity and drug dependence. The effects of chronic nicotine, nicotine withdrawal and the responsivity to nicotine following a sustained period of abstinence were examined utilising a longitudinal design. To mimic more precisely to the pattern of nicotine intake in smokers, nicotine was administered chronically via osmotic mini pumps. Administration of nicotine in such a manner enabled the maintenance of plasma levels of nicotine at approximately $\sim 44\text{ng ml}^{-1}$, a level which is similar to that of smokers who consume 30 cigarettes per day (Murrin, Ferrer, Zeng and Hayley, 1987; Benowitz 1988). Evidence suggests that smokers attempt to maintain a constant blood level of nicotine, through varying both the quantity of cigarettes consumed and actual smoking behaviour (e.g. Chait and Griffiths, 1982; McMorro and Foxx 1983; Chait, Ross and Griffiths, 1985). This cannot be accurately mimicked by single daily test injections of nicotine, which additionally increases the need for handling and induces greater stress in the animal. The one disadvantage of osmotic pumps, is that nicotine continues to be released during periods of sleep, when human smokers would not normally be exposed to nicotine. However, the substantial advantages of osmotic mini pump methodology clearly outweighs this drawback.

Chronic nicotine led to an increase in both impulsive choice and disinhibition. The effect on impulsive choice appeared be restricted to low “trait” impulsive animals, with high impulsive animals displaying limited sensitivity to chronic nicotine. A comparable finding across measures of impulsivity was that the greatest nicotine induced effect was observed during the initial stages of drug treatment, following which a transient strengthening in self control was observed. This pattern of effect was most likely a reflection of the development of tolerance to nicotine, although this was not supported statistically in either paradigm. The inactivation and up-regulation of nAChRs associated with longer term exposure to nicotine, is likely to have mediated the less substantial effects of nicotine on impulsivity with the progression of treatment (Collins et al., 1990; Wonnacott, 1990; Littleton, 2001). As both components of impulsivity demonstrated evidence of tolerance to nicotine, these findings provide some indirect support that each may be mediated by comparable nicotinic subtypes. The $\alpha 4\beta 2$ and $\alpha 7$ receptors subunits, which display the longest duration of inactivation and greatest up-regulation during chronic nicotine exposure, are likely candidates (Olale et al., 1997; Quick and Lester, 2002; Nguyen, Ramussen and Perry, 2003). Interestingly, these are the receptor subunits that have been previously implicated in behavioural disinhibition (Blondel et al., 2000; Grottick and Higgins, 2000; Keller et al., 2005; Hoyle et al., 2006).

The induced increase in impulsivity during chronic exposure to nicotine supports not only previous findings of the effects of chronic nicotine in paradigms of inhibitory control and impulsive choice, but also previous reports of the effects of chronic cocaine and amphetamine (Richards et al., 1999a; Blondel et al., 2000; Jentsch et al., 2002; Paine et al., 2003; Dallery and Locey, 2005). Inconsistent with the present findings is the lack of effect of chronic cocaine in the go/no-go task reported by Paine et al. (2003). However, the reward contingencies in the go/no-go task differed in Paine et al. study, in that responding during No-go trials did not lead to the loss of reinforcement. Exploring the effects of chronic cocaine in a symmetrically reinforced go/no-go paradigm, as utilised in the present research, may have yielded different results.

The demonstration that chronic drug regimes that model the pattern of drug use in dependent users more precisely, lead to an increase in impulsivity leaves little doubt that chronic drug exposure renders abusers more impulsive. With increasing drug exposure however, tolerance to the drug's influence on impulsivity is likely to develop. These findings suggest once again that drug induced impulsivity may play an important role during the early stages of addiction.

9.4. THE EFFECTS OF NICOTINE WITHDRAWAL ON DISINHIBITION AND IMPULSIVE CHOICE

The termination of treatment caused a spontaneous nicotine abstinence syndrome, that based on aggregated behavioural signs, continued for approximately 85 hours in both groups of animals in Experiment 4 and 7 (Malin et al., 1992; Hildebrand et al., 1997; 1999; Epping-Jordan et al., 1998; Watkins et al., 2000a; Harrison et al., 2001; Malin et al., 2006). Initial nicotine induced displayed contrasting effects on each subcomponent of impulsivity. On inhibitory control, a rebound increase in inhibitory control was observed, an effect which was at its maximum at 12 and 60 hours post cessation of drug treatment. In contrast, low "trait" impulsive animals displayed a continued level of heightened impulsive choice following termination of nicotine exposure. Impairments in attention and memory, alterations in primary motivation and diminished sensitivity to reward could not account for the increase in inhibitory control in the go/no-go task. Therefore, strong support was provided that the data reflected a genuine decrease in impulsive disinhibition during the early stages of nicotine withdrawal (Epping-Jordan et al., 1998; Watkins et al., 2000b; Harrison et al., 2001; Cryan et al., 2003; Shoiab and Bizarro, 2005; see also findings of Chapter 3).

At first glance it may appear surprising that opposing effects of nicotine deprivation occur on impulsive choice and disinhibition. However, examination of the literature indicates that these two subcomponents of impulsivity have often demonstrated dissociable relationships with addiction at both the behavioural and neurobiological level (e.g. Reynolds et al., 2006a; Talpos

et al., 2006). For example, lesions to the STN, decreased impulsive choice in a delayed reward task whilst increasing disinhibited responding in the DRL (Winstanley et al., 2005; Uslaner and Robbins, 2006). Similarly, damage to the ACC, PLC and ILC elicits profound disinhibition whilst having a minimal impact on preference of immediate over delayed gratification (e.g. Cardinal et al., 2001; Christakou et al., 2004; Chudasama et al., 2004; Picton et al., 2007). Furthermore, the subcomponents of impulsivity have been shown to be dissociated at the receptor subtype level in both serotonergic and dopaminergic systems (Wade et al., 2000; Talpos et al., 2006; van Gaalen et al., 2006). As these neurobiological mechanisms are also arguably involved in the mediation of nicotine withdrawal, it is therefore likely that they may also be playing a role in modulating the opposing effects of initial withdrawal on each of these subcomponents of impulsivity in the present research (e.g. Fung et al., 1996; Hildebrand et al., 1999; Watkins et al., 2000a; Koob and Le Moal, 2001; Kenny and Markou, 2004; Rahman et al., 2004).

It is important to highlight the finding that the high impulsive “trait” animals in the model of delayed reward displayed evidence of a decrease in impulsive choice that was more comparable to the increase in inhibitory control in the go/no-go task. However, due to the low subject numbers and extensive variability in performance in the high impulsive animals it is difficult to make valid comparisons at this stage. It is therefore essential that future research explores further the difference in response of high and low impulsive animals at this stage of withdrawal.

In contrast, the second week of withdrawal was associated with both heightened impulsive choice and evidence of disinhibited responding. This finding, that dysfunction in inhibitory control and decision making persisted in the absence of nicotine, greatly supports the theory that neural changes that arise during chronic drug exposure render subjects highly impulsive in the long term. The report that abstinent smokers display continued abnormalities in regions of the PFC that are highly implicated in both behavioural and cognitive impulsivity provides some support for this interpretation (Gallinat et al., 2006; Neuhaus et al., 2006). With longer term abstinence a strengthening of self control was observed, suggesting that the nicotine induced effects on impulsivity are temporary and transient. At 21 days post termination of treatment levels of both inhibitory control and impulsive choice had returned to pre-drug levels of impulsivity. To date, human research has been unable to elucidate whether the observed reduction in levels of both inhibitory control and impulsive choice in ex-smokers can be attributed to either a pre-existing trait that had enabled these individuals to successfully abstain, or instead, reflect a strengthening of behavioural control following smoking cessation (Bickel et al., 1999; Yakir et al., 2007). The present data provide strong support for the latter of these interpretations. However, it should be emphasised that these findings do not imply that trait impulsivity did not also aid successful abstinence. Indeed, recent research has indicated that

both heightened sensitivity to delayed reward and poor inhibitory control significantly predict relapse in smokers (Dallery and Raiff, 2007; Krishnan-Sarin et al., 2007; Yoon et al., 2007).

Interestingly, evidence of a reduction in heightened impulsive choice following abstinence has also been demonstrated in ex-users of heroin, alcohol and methamphetamine (Bretteville-Jensen, 1999; Petry, 2001; Kirby and Petry, 2004; Bornovalova et al., 2005). Based on the present findings it could be tentatively argued that the induced impulsive choice in dependent users of other pharmacological classes of drugs may also be a reversible drug effect. In contrast, chronic cocaine abuse has been associated with no evident reduction in sensitivity to delayed reward (Kirby and Petry, 2004; Bornovalova et al., 2005; Heil et al. 2006). The lack of reduction following abstinence in this population of abusers may suggest that the neural adaptations associated with chronic cocaine exposure may be more severe. As a result, a greater time period might be required to recover and mediate the strengthening of self control (Lyyo et al., 2004). In support of this argument, abnormalities in regions of the PFC have been found to persist for months following abstinence in cocaine abusers, attributed in part to a down-regulation of D2 receptors (Volkow et al., 1993; 1997).

This argument may also explain the continued impaired levels of inhibitory control also observed in abstinent cocaine and alcohol users (Bjork et al., 2004; Goudriaan et al., 2005; Verdejo-Garcia et al., 2007). It should, however, be acknowledged that many of these studies did not control for smoking behaviour in abstinent drug abusers. Based on the present findings, it could be argued that nicotine may contribute to the continued dysfunctional levels of impulsivity observed in past research.

Taken together, these findings suggest that both disinhibition and impulsive choice could be fundamental processes underlying relapse during withdrawal. Sensitivity to delayed gratification, however, may play a more prominent role during early drug deprivation. Interestingly, the greatest percentage of smokers relapse in the first two weeks of abstinence (Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995), a time period during which, according to the present research, impulsivity appears to be at its greatest. During this time period, individuals are likely to display less control over drug seeking and taking behaviour, whilst delayed health and social benefits associated with a drug free life style will be discounted to the greatest degree. When combined these effects are likely to render the individual highly vulnerable to relapse.

9.5. ALTERATIONS IN RESPONSIVITY TO NICOTINE FOLLOWING A SUSTAINED PERIOD OF ABSTINENCE

Although early stages of abstinence are associated with the highest rates of relapse, longer term vulnerability after 'quitting' is well documented (Stephens and Cottrell, 1972; Robinson and Berridge, 2000; 2003; Hughes et al, 2004; Piasecki, 2006; Hser, 2007). One of the greatest predictors of long term relapse in smokers is the lapse of smoking a single cigarette (Brandon et al., 1990; Nides et al., 1995; Shiffman et al., 1996). In order to determine the possible role of impulsivity in longer term relapse assessment was made of the alteration in response to nicotine following a sustained period of abstinence. Acute administration of nicotine, in both paradigms, increased impulsivity in drug naïve animals in a manner that directly replicated the finding of Chapter 3 and 6. In the delayed reward task previous nicotine exposure had no effect on the subsequent response to acute nicotine. Nicotine increased levels of impulsive choice in a dose dependent manner in both saline and nicotine treated animals. Conversely, previous nicotine exposure led to a profound hypersensitivity of disinhibition in response to acute nicotine administration. Animals chronically treated with nicotine displayed a behaviourally selective nicotine induced loss of control across all doses tested (0.125, 0.25 and 0.5mg/kg), whilst nicotine naïve animals displayed a reduction in inhibitory control only at the highest dose.

These findings are the first to demonstrate that chronic nicotine can render subjects sensitised to the effects of nicotine on disinhibition after a period of sustained abstinence. Unlike previous research, strong evidence was presented that the enhanced disinhibited responding was not due to secondary changes in locomotor activity (Clarke and Kumar, 1983; Chaudhry, Turanis and Karler, 1988; Pierce and Kalivas, 1997; Grottick and Higgins, 2000; 2001). These findings have provided crucial evidence for the role of inhibitory control in smoking relapse. More specifically, an abstaining smoker that is exposed to even low levels of nicotine is at a substantial risk of relapsing due to the profound drug induced loss of control over future drug seeking and taking behaviour. Substantial research has demonstrated that the mesocorticolimbic DA system becomes hypersensitive to the effects of nicotine following chronic exposure (Robinson and Berridge, 1993). This in turn leads to the augmented release of DA in the NAc (Cadoni and Di Chiara, 2000; Olausson et al., 2001a; Rahman, Zhang and Corrigall, 2003) and PFC (Vezina et al., 1992; Nisell et al., 1996) following subsequent exposure to nicotine. As DA receptor activation appears to be crucial for the effects of stimulant drug induced disinhibition, it is therefore likely that alterations in dopaminergic systems plays an important role in these effects (Van Gaalen et al., 2006; Pattij et al., 2007).

The contrasting lack of increase in responsivity to the effects of nicotine on impulsivity once

again supports the theory that impulsive choice and disinhibition are separate and dissociable components of impulsivity (Everden, 1999). These findings do not preclude the possibility that impulsive choice might also play a role in long-term relapse. Indeed, impulsive choice still increased profoundly following nicotine administration. Rather, these data indicate that a loss of inhibitory control may play more of a key role in the mediation of drug relapse following smoking during a “quit” attempt. Interestingly, high trait impulsive animals displayed evidence of a contrasting tolerance to nicotine following chronic exposure. Whilst no firm conclusions can as yet be made, the recent evidence indicating a possible blunted DA response in high impulsive individuals in response to stimulant drugs, is likely to have in part mediated this effect (Oswald et al., 2007).

9.6. IMPLICATIONS FOR THEORIES OF DRUG ADDICTION

In introducing this thesis, drug addiction was identified as the uncontrollable compulsive pattern of drug-seeking and drug-taking behaviour that takes place at the expense of most other activities and in the face of damaging health and social consequences (Robinson and Berridge, 1993; West, 2006). This definition highlights the two major characteristics of drug addictive disorders; compulsion and the loss of control over drug intake (Koob and Le Moal, 2001; 2007).

Compulsion is characterised in humans by intense and involuntary drug craving believed to lead to the desire to want to repeatedly experience the reinforcing properties of the drug of abuse (Markou et al., 1993; Robinson and Berridge, 1993; 2003). It is believed that compulsive drug seeking and taking behaviour arises due to the neuroadaptations in the mesolimbic DA system that renders individuals sensitised to both the drug of abuse and drug related stimuli (Robinson and Berridge, 2000; 2003). This is plausible for nicotine although this compound was not considered in Robinson and Berridge’s theory. Repeated drug use leads drug related cues to acquire incentive value making them increasingly ‘wanted’, thus giving rise to intense craving and the development of drug seeking and taking in their presence. The influence that these cues exert over behaviour is believed to be mediated by interactions between the mesolimbic DA system and basolateral amygdala (Everitt et al., 1999).

Despite extensive knowledge of the neurobiological and behavioural mechanisms underlying compulsive drug use, the role of impulsivity in drug addiction is only now beginning to unfold. Whilst the mesolimbic-amygdala neural systems are believed to mediate the compulsive aspects of addiction, regions of the PFC have instead been implicated in the modulation of impulsivity (Jentsch and Taylor, 1999). Although more recent theoretical accounts of addiction are beginning to acknowledge the importance of impulsivity in drug addiction (Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Lubman et al., 2004) the precise role of impulsivity in the establishment, maintenance and relapse of the disorder is unclear.

The present thesis provides strong evidence to suggest that drug induced impulsivity is likely to be critically involved in both the initial and end stages of addiction (Dawe et al., 2004). More specifically, when drug naïve individuals are first exposed to a drug of abuse, such as nicotine, this will lead to a substantial loss of control over future drug seeking and taking behaviour. Furthermore, a heightened sensitivity to delayed gratification will be induced. As a consequence the individual will be more likely to select the more immediate reinforcing effects of the drug over the larger, often delayed social and health benefits associated with a drug free lifestyle. The induced behavioural and cognitive impulsivity at this early stage is likely to lead to further drug taking behaviour and increase and promote the transition to addiction.

The data of the present thesis provides evidence that with repeated drug exposure, tolerance to drug effects on impulsive choice and disinhibition may rapidly develop. This suggests that at least in terms of drug induced impulsivity, this may play a minimal role in the continued maintenance of addiction, suggesting that perhaps the more compulsive features of the disorder maintain the cycle of addiction at this stage (Koob and Le Moal, 2001). However, following the termination of drug use, the role of impulsivity appears to become more important once again. Impulsive choice and disinhibition may however be differentially involved at the end stage of addiction. Both initial and longer term drug withdrawal appears to be associated with a heightening of impulsive choice. The long term rewards associated with continued abstinence will, as a consequence, be discounted to a greater degree at this stage. Individuals are therefore more likely to select the immediate reinforcing effects of the drug or relief of withdrawal resulting in drug relapse.

Disinhibition, in contrast, whilst playing a minimal role during the early stages of drug withdrawal appears to be a key aspect underlying relapse during longer term abstinence. The neural adaptations associated with chronic drug exposure appear likely to render individuals hypersensitive to the drug induced effects on disinhibition. Exposure to the drug at this stage would therefore be detrimental, as it could lead to a substantial loss of control over drug taking behaviour and subsequent relapse. Although the neurobiological mechanisms were not evaluated in the present research it is highly likely that the sensitisation of the mesocorticolimbic DA system is mediating the increased responsivity to nicotine on behavioural disinhibition (Robinson and Berridge, 1993; Cadoni and Di Chiara, 2000; Olausson et al., 2001a; Rahman, Zhang and Corrigall, 2003). As sensitisation of this DA system is believed also to mediate the compulsive aspect of addiction, it may be at this end stage of abstinence that the two distinct traits come to interact to continue the cycle of addiction (Jentsh and Taylor, 1999; Koob and Le Moal, 2001; Goldstein and Volkow, 2002).

The establishment of the importance of impulsivity at these stages of addiction warrants the need for future pharmacological and behavioural treatments to focus upon both increasing tolerance to delay and behavioural control. This argument is further supported by increasing research suggesting that both high levels of intolerance to delayed reward and disinhibition predicts drug relapse (Moeller et al., 2001; Dallery and Raiff, 2007; Krishnan-Sarin et al., 2007; Yoon et al., 2007). For example, both contingency management and motivational enhancement programmes that focus upon developing skills necessary to improve self control may prove to be successful (Higgins et al., 1986; Higgins et al., 2000). Pharmacological treatments that have demonstrated evidence of reducing impulsivity, such SSRIs and D1 and D2 receptor antagonists, may also transpire to be effective treatment strategies, either alone or in combination with behavioural treatments (Richards et al., 1993; Schmitz et al., 1998; Killen et al., 2000; Wolff and Leander, 2002; van Gaalen et al., 2006; Pattij et al., 2007).

9.7. FUTURE DIRECTIONS

The main findings of this thesis suggest that the heightened impulsivity in smokers is, in part, a consequence of nicotine exposure. This however does not suggest that the relationship between impulsivity and drug dependence is unidirectional. Accumulating evidence in both the human and animal literature suggests that trait levels of impulsivity may predict future drug use, suggesting that impulsivity may be both a risk factor and a consequence of addiction (Poulos et al., 1995; Tarter et al., 2003; Perry et al., 2005; Dalley et al., 2007). Therefore, in order to gain a complete understanding of the nature of the relationship between nicotine addiction and impulsivity, future research should now focus upon investigating the role of disinhibition and impulsive choice as potential predictors of nicotine dependence.

Although genetics are likely to play a role in individual differences in trait impulsivity, interest is increasingly focussing upon how non-genetic, environmental factors may also influence levels of impulsivity, and in turn, increase the vulnerability to drug abuse. For example, adverse early life experiences in animals, such as rearing in social isolation, has been found to lead to increased disinhibition and alcohol intake (Higley and Linnoila, 1997; Hall, 1998). Conversely, rearing rodents in isolation has led to *lower* levels of impulsive choice being displayed (Hellemans et al., 2005). Taken together, these findings suggest that early adverse experiences may have dissociable effects on differing components of impulsivity. What this research importantly highlights is that additional factors may influence the nature of the relationship between impulsivity and addiction, and future research needs to acknowledge these to further our understanding.

Whilst the present thesis has increased our understanding of the relationship between impulsivity and drug dependence at a behavioural level, it is crucial that future research begins

to elucidate the neurobiological processes that may be involved. Throughout the chapters of this thesis, tentative suggestions have been made regarding the possible neurobiological events that may be modulating the alterations in impulsivity and these may provide the preliminary basis for future research. Such investigations are essential if future pharmacological treatments are to be developed that target increasing self-control in the hope of aiding successful abstinence.

Finally, a limitation of the research within this thesis is that the drug of abuse was passively administered by the experimenter, instead of being self-administered, as is the case for real life drug taking behaviour. Future research that investigates both the effects of chronic self administration on impulsivity and withdrawal from drug self administration may provide a more sophisticated model to assess the relationship between impulsivity and drug addiction.

9.8. CONCLUSIONS

Studies reported in this thesis have increased greatly our understanding of the complex relationship between drug addiction and impulsivity. The robustness of the data has left little room for doubt that the heightened impulsive choice and disinhibition in smokers is in part a consequence of nicotine exposure. The findings suggest that drug induced impulsivity may be a fundamental mechanism involved in both the initial and end stages of addiction. More specifically, impulsive choice and disinhibition is increased following both acute and early stages of chronic drug treatment, which may lead to the loss of control over future drug seeking and taking behaviour and the selection of the more immediate reinforcing properties of drugs of abuse. Following cessation of treatment a sustained heightened level of impulsive choice is observed in low "trait" impulsive animals, that gradually returns to pre-drug BL levels following a three week period. Conversely, initial drug deprivation is associated with a short-lived rebound increase in inhibitory control, following which an increase in disinhibition is observed during longer term withdrawal. Despite nicotine induced effects on inhibitory control diminishing 21 days following termination of treatment, subjects remained hypersensitive to nicotine's effects on impulsivity. Together these findings provide strong support for the role of impulsivity in drug relapse during the first two weeks of abstinence. This is especially important given indications that abstinent abusers are most vulnerable to relapse during this period (Gossop et al., 1989; Garvey et al., 1992; Hughes et al., 1992; Kenford et al., 1994; Law and Tang, 1995). The demonstration that chronic nicotine exposure renders animals hypersensitive to the effects of nicotine following a sustained period abstinence, further indicates that disinhibition is likely to be a fundamental process mediating longer term vulnerability to drug relapse. It is essential that future research continues to address the role of impulsivity in drug addiction, and more importantly the neurobiological mechanisms mediating the relationship. Such research should improve both our understanding of, and our ability to treat the debilitating disorder of drug addiction.

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APPENDICES

APPENDIX 1: EXPERIMENT 2 A-C

Appendix Table 1.1: Acute Effects of Nicotine on Choice Behaviour

Behavioural Measure	Main effect dose	Main effect delay	Dose x delay interaction
Overall % choice of delayed reward	F (3,30) = 30.478, p<0.001	N/A	N/A
% choice x delay	F (3, 30)= 25.610, p<0.001	F (1.653, 16.528) = 74.188, p<0.001	F (12,120) = 5.016, p<0.001

Appendix Table 1.2: Simple Effects Analysis of Nicotine Dose X Delay Interaction: Main Effects of Dose at Each Delay Level

Delay level	Main effect dose
Zero seconds	n.s.
Ten seconds	F(3, 30) =6.550, p=0.002
Twenty seconds	F(3, 30) =17.236, p<0.001
Forty seconds	F(3, 30) =26.187, p<0.001
Sixty seconds	F(3, 30) =10.592, p<0.001

Appendix Table 1.3: Simple Effects Analysis of Nicotine Dose X Delay Interaction: Main Effects of Delay at Each Dose Level

Dose level	Main effect delay
0 mg/kg	F(4,36) = 34.477, p<0.001
0.125 mg/kg	F(4,36) = 48.030, p<0.001
0.25 mg/kg	F(4,36) = 41.156, p<0.001
0.5 mg/kg	F(4,36) = 60.880, p<0.001

Appendix Table 1.4: Acute Effects of Nicotine on Speed of Responding

Behavioural Measure	Main effect dose
Initiation of trial latency	$F(3,30) = 8.0101, p < 0.001$
Immediate choice response latency	n.s.
Delayed choice response latency	n.s.
Immediate choice magazine response latency	n.s.
Delayed choice magazine response latency	$X^2 = 11.182, df = 3, p = 0.011$

Appendix Table 1.5: Acute Effects of Nicotine on Omissions

Behavioural Measure	Main effect dose
Trial initiation omissions	n.s.
Delayed choice magazine omissions	$X^2 = 9.720, df = 3, p = 0.021$

Appendix Table 1.6: Acute Effects of Mecamylamine on Choice Behaviour

Behavioural Measure	Main effect dose	Main effect delay	Dose x delay interaction
Overall % choice of delayed reward	$F(3,27) = 4.5.8, p = 0.011$	N/A	N/A
% choice x delay	$F(3,37) = 3.246, p = 0.037$	$F(2.055, 18.493) = 31.101, p < 0.001$	$F(12,108) = 2.098, p = 0.023$

Appendix Table 1.7: Simple Effects Analysis of Mecamylamine Dose X Delay Interaction: Main Effects of Dose at Each Delay Level

Delay level	Main effect dose
Zero seconds	n.s.
Ten seconds	n.s.
Twenty seconds	$F(3,27) = 4.063, p = 0.017$
Forty seconds	n.s.
Sixty seconds	n.s.

Appendix Table 1.8: Simple Effects Analysis Of Mecamylamine Dose X Delay Interaction: Main Effects Of Delay At Each Dose Level

Dose level	Main effect delay
0 mg/kg	$F(4,36) = 19.678, p < 0.001$
0.1 mg/kg	$F(4,36) = 16.241, p < 0.001$
0.3 mg/kg	$F(4,36) = 19.678, p < 0.001$
1.0 mg/kg	$F(4,36) = 26.390, p < 0.001$

Appendix Table 1.9: Acute Effects of Mecamylamine on Speed of Responding

Behavioural Measure	Main effect dose
Initiation of trial latency	n.s.
Immediate choice response latency	n.s.
Delayed choice response latency	n.s.
Immediate choice magazine response latency	n.s.
Delayed choice magazine response latency	n.s.

Appendix Table 1.10: Acute Effects of Mecamylamine on Omissions

Behavioural Measure	Main effect dose
Trial initiation omissions	n.s.
Delayed choice magazine omissions	n.s.

Appendix Table 1.11: Acute Effects of Combination Treatment on Choice Behaviour

Behavioural Measure	Main effect dose	Main effect delay	Dose x delay interaction
Overall % choice of delayed reward	F (4,26) = 17.487, p<0.001	N/A	N/A
% choice x delay	F (4, 36)= 20.248, p<0.001	F (2.315, 20.839) = 59.861, p<0.001	F (12,120) = 2.317, p=0.005

Appendix Table 1.12: Simple Effects Analysis of Combination Treatment Dose X Delay Interaction: Main Effects of Dose at Each Delay Level

Delay level	Main effect dose
Zero seconds	n.s.
Ten seconds	F(4, 36) =5.959, p=0.001
Twenty seconds	F(3, 36) =10.079, p<0.001
Forty seconds	F(3,36) = 12.676, p<0.001
Sixty seconds	F(4,36) = 4.742, p=0.004

Appendix Table 1.13: Simple Effects Analysis of Combination Treatment Dose X Delay Interaction: Main Effects of Delay at Each Dose Level

Dose level (mecamylamine / nicotine)	Main effect delay
0 mg/kg / 0 mg/kg	F(4,36) = 34.542, p<0.001
0 mg/kg / 0.5 mg/kg	F(4,36) = 23.101, p<0.001
0.1 mg/kg / 0.5 mg/kg	F(4,36) = 28.563, p<0.001
0.3 mg/kg / 0.5 mg/kg	F(4,36) = 29.076, p<0.001
1.0 mg/kg / 0.5 mg/kg	F(4,36) = 26.865, p<0.001

Appendix Table 1.14: Acute Effects of Combination Treatment on Speed of Responding

Behavioural Measure	Main effect dose
Initiation of trial latency	F(4,36)= 4.788, p=0.003
Immediate choice response latency	n.s.
Delayed choice response latency	n.s.
Immediate choice magazine response latency	n.s.
Delayed choice magazine response latency	n.s.

Appendix Table 1.15: Acute Effects of Combination Treatment on Omissions

Behavioural Measure	Main effect dose
Trial initiation omissions	n.s.
Delayed choice magazine omissions	n.s.

APPENDIX 2: EXPERIMENT 6 A-C

Appendix Table 2.1: Acute Effects of Nicotine on Accuracy of Responding

Behavioural Measure	Main effect dose
Total % Correct Trials	$F(4,44) = 5.769, p=0.001$
% Correct Go Trials	$F(4,44) = 12.264, p=0.000$
% Correct No-go Trials	$F(4,44) = 2.802, p=0.037$

Appendix Table 2.2: Acute Effects of Nicotine on Anticipatory Responding

Behavioural Measure	Main effect dose
No. of Go trials with Early Responses	$F(4,44) = 9.368, p<0.001$
No. of No-go trials with Early Responses	$F(4,44) = 3.187, p=0.022$
No. of Go trials with Magazine Entries	$X^2 = 15.159, df= 4, p=0.004$
No. of No-Go trials with Magazine Entries	n.s.

Appendix Table 2.3: Acute Effects of Nicotine on Speed of Responding

Behavioural Measure	Main effect dose
Correct Response Latency	$F(4,44) = 6.203, p<0.001$
Incorrect Response latency	n.s.
Go magazine Latency	n.s.
No-go Magazine Latency	n.s.

Appendix Table 2.4: Acute Effects of Mecamylamine on Accuracy of Responding

Behavioural Measure	Main effect dose
Total % Correct Trials	n.s.
% Correct Go Trials	n.s.
% Correct No-go Trials	n.s.

Appendix Table 2.5: Acute Effects of Mecamylamine on Anticipatory Responding

Behavioural Measure	Main effect dose
No. of Go trials with Early Responses	n.s.
No. of No-go trials with Early Responses	n.s.
No. of Go trials with Magazine Entries	n.s.
No. of No-Go trials with Magazine Entries	$F(3,30) = 3.882, p=0.019$

Appendix Table 2.6: Acute Effects of Mecamylamine on Speed of Responding

Behavioural Measure	Main effect dose
Correct Response Latency	n.s.
Incorrect Response latency	$F(3,30) = 5.577, p=0.004$
Go magazine Latency	n.s.
No-go Magazine Latency	n.s.

Appendix Table 2.7: Acute Effects of Combination Treatment on Accuracy of Responding

Behavioural Measure	Main effect dose
Total % Correct Trials	$F(4,40) = 18.761, p<0.001$
% Correct Go Trials	n.s.
% Correct No-go Trials	$F(4,40) = 20.512, p<0.001$

Appendix Table 2.8: Acute Effects of Combination Treatment on Anticipatory Responding

Behavioural Measure	Main effect dose
No. of Go trials with Early Responses	n.s.
No. of No-go trials with Early Responses	n.s.
No. of Go trials with Magazine Entries	n.s.
No. of No-Go trials with Magazine Entries	n.s.

Appendix Table 2.9: Acute Effects of Combination Treatment on Speed of Responding

Behavioural Measure	Main effect dose
Correct Response Latency	n.s.
Incorrect Response latency	$F(4,40) = 5.694, p=0.001$
Go magazine Latency	n.s.
No-go Magazine Latency	n.s.

APPENDIX 3: EXPERIMENTS 7 & 8

Appendix Table 3.1: Individual Rat Data, Indicating Steepness of Discounting (K) and Goodness of Fit (R²)

Subject	Treatment Group	k value	R ² value	High or Low "Trait"
1	N	0.39	0.89	L
2	N	0.16	0.87	L
3	N	0.42	0.81	H
4	N	0.42	0.92	H
5	N	0.47	0.87	H
6	N	0.27	0.70	L
7	N	0.13	0.64	L
8	N	0.29	0.97	L
9	N	0.38	0.95	L
10	N	0.11	0.83	L
11	N	0.15	0.96	L
12	N	0.95	0.72	H
13	N	0.08	0.88	L
14	S	0.23	0.88	L
15	S	0.84	0.88	H
16	S	0.16	0.95	L
17	S	0.40	0.93	H
18	S	0.50	0.94	H
19	S	0.48	0.99	H
20	S	0.38	0.71	L
21	S	0.40	0.75	H
22	S	0.04	0.55	L
23	S	0.21	0.94	L
24	S	0.31	0.63	L